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

T. Stemeseder1, E. Klinglmayr1, S. Moser2,3, L. Lueftenegger4,5, R. Lang4, M. Himly1, G. J. Oostingh5, J. Zumbach2, A. C. Bathke6, T. Hawranek4 & G. Gadermaier1

1Department of Molecular Biology; 2School of Education, University of Salzburg, Salzburg, Austria; 3TUM School of Education, Technical University of Munich, Munich, Germany; 4Department of Dermatology, Paracelsus Medical University Salzburg; 5Biomedical Sciences, Salzburg University of Applied Sciences, Puch; 6Department of Mathematics, University of Salzburg, Salzburg, Austria


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 nonselected 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 11 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.

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 geographic 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 and 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 with 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 nonselected 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/169/6-2013. 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 μl 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 ≥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, and contingency data were 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 Fig. S1. To assess associations between ISU to single allergens and allergen, 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 (Fig. S2).

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, that is, PR-10, grass group 1, profilins, and Ole e 1-like proteins ($P < 0.01$) compared with 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 with multireactors.

Figure 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 $N$.

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 ($n = 82/142$ 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 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).

© 2016 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd
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 with 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 monoreactive 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 (ISU ≥0.3) at least once in the set of 112 components. Molecules are grouped by allergen source.

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* ($r > 0.933$), and high correlation between group 1 and 2 mite allergens ($r > 0.602$). As anticipated, very high to moderate correlations ($r > 0.5$) were also found for allergens of the same protein family, for example, profilins, PR-10, grass group I allergens, polcalcins, and lipocalins. Similar values ($r > 0.527$) were interestingly also observed for nonrelated allergens of grass pollen. Sensitization frequency as well as ISU levels within the PR-10 family (Fig. 5) were highest with
Figure 3 Set diagrams showing the IgE sensitization profile of the study population. (A) All allergen sources; (B) pollen allergen sources grouped by source; (C) grass pollen allergens; (D) tree pollen allergens; (E) weed pollen allergens; (F) Ole e 1-like allergens. Only groups containing three or more positive IgE samples are depicted; circle size approximately represents number of subjects.
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 ($P < 0.05$, $q > 0.2$), 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 ($P < 0.001$, $q = 0.584$), and Pla a 2 ($P < 0.001$, $q = 0.481$). 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 ($P < 0.05$, $q = -0.142$).

IgE sensitization and allergies

In order to relate IgE sensitization with allergic symptoms, subjects reported doctors’ diagnosed and self-reported allergies 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 with 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 the 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.

Figure 4  Set diagrams showing IgE sensitization profiles to specific allergen sources. (A) Food allergens; (B) mite allergens; (C) insect venom allergens. Only groups containing three or more positive IgE samples are depicted; circle size approximately represents number of subjects.

Bet v 1, followed by Cor a 1.0010, 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.
This study analyzed IgE sensitization profiles of nonselected pupils from the region of Salzburg, Austria, by means of the ImmunoCAP ISAC®. Sensitization data were 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- to 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 confirms 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 toward more diverse and higher levels of allergen sensitizations following the epidemiologic 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 six 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 a 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 with 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 with 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 with the Czech cohort with 9.3% (15). On the other hand, allergens from cypress and juniper pollen seem to play a neglected 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 1 1, among pollen-sensitized individuals in our study. Sensitization profiles of Pla 1 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 plays 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 United States 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 multisensitized individuals. This might be due to the spread from major allergens toward 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 multisensitized 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 seems to be rather independent from other sources and interestingly, and IgE levels to Der p 1/2 were not elevated in multisensitized 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 allergies 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 gastrointestinal tract (including intolerances), dermatological reactions, or nonallergic 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, that is, 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, for example, Pla l 1 or Ole e 1. Such cohort studies provide a deep insight into IgE sensitization profiles, enabling the monitoring of populations over time, and can reveal prognostic molecules before onset of the disease (29). As 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).

Acknowledgments
Carmen Wageneder-Schmid and the Biomedical Sciences team of the Salzburg University of Applied Sciences (K. Schwenoha, L. Helminger, J. Gmachl-Baumgartner, R. Wilsche, U. Fötischl) are acknowledged for their support regarding study design and carrying out the blood sampling, respectively. Further we thank the teachers E. Oberkofler (HBLA Ursprung), M. Hauer (BG Tamsweg), S. Fischinger (NMS Bürmoos), K. Schaffer (PüC BORG Radstadt), B. Wanzenböck (Musisches Gymnasium Salzburg), J. Pöttler (BG Seekirchen), R. Pilotto-Koller and P. Paraschini-Wolfberger (PG St. Rupert), M. Anderluch (BHAK/BHAS I Salzburg) and G. Mussill and L. Mackner-Rath (BORG Nomtal), and the pupils of the participating schools for supporting the project.

Author contributions
Gabriele Gadermaier, Martin Himly, Thomas Hawranek, and Joerg Zumbach conceived the study. Teresa Stemeseder, Eva Klinglmayr, Stephanie Moser, Lisa Lueftenegger, and Gertje J. Oostingh performed experiments. Teresa Stemeseder, Arne C. Bathke, and Roland Lang analyzed data and calculated statistics. Teresa Stemeseder and Gabriele Gadermaier wrote the manuscript.

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

Supporting Information
Additional Supporting Information may be found in the online version of this article:
Figure S1. Set diagrams were depicted according to groupings of allergens.
Figure S2. Sensitization prevalence and ISU levels of selected single allergens.

References

© 2016 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd
27. De Amici M, Ciprandi G. The age impact on serum total and allergen-specific IgE. Allergy and Clinical Immunology; 2014: pp 112–114.