

ORIGINAL ARTICLE

Molecular and clinical characterization of nut, peanut, and seed allergies: the role of seed storage proteins

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Summary

Background. Seed Storage Proteins (SSPs) associate with severe allergic reactions to tree nuts, peanuts, and seeds. This study aimed to characterize the molecular and clinical profiles of SSP sensitization, in patients allergic to peanuts, tree nuts, and/or seeds and sensitized to 2S Albumins (2S) or 7S and 11S Globulins (7S and 11S, respectively).

Methods. A retrospective analysis from 2016 to 2024 of the characteristics of peanuts, and/or seeds allergic patients who were sensitized to SSPs (identified by ImmunoCAPTM ISAC) was performed. Reaction severity was graded using the oFASS scale. **Results.** Sixty-six patients (median age 10 years, range 1–67, IQR 15) were included. Walnut caused 41% of the reactions, followed by hazelnut and peanut (21%). Sensitization rates were 88% for 2S (Jug r 1 48%, Ana o 3 29%), 36% for 11S (Cor a 9 29%), and 23% for 7S. Co-sensitization occurred within and across protein families ($p < 0.05$). Jug r 1, Cor a 14, Ana o 3, Ara h 1, 2, and 6 were clinically relevant for walnut, hazelnut, cashew, and peanut allergies ($p < 0.05$). Severe reactions correlated with co-sensitization to non-specific lipid transfer proteins (nsLTPs) ($p = 0.05$). **Conclusions.** Jug r 1 (the main sensitizer), Cor a 9, Ana o 3, Ara h 1, 2, and 6 appear to be good markers of nut/peanut allergy. Co-sensitization to nsLTPs seems to exacerbate reaction severity. Identifying

SSP sensitizations can improve diagnosis, management, and personalized recommendations for allergic patients.

Key words

Nut; peanut and seed allergy; seed storage proteins; cross-reactivity; food allergy; anaphylaxis.

Impact statement: This study advances the understanding of tree nut, peanut, and seed allergies, highlighting seed storage proteins, bridging clinical and molecular gaps to improve diagnosis, treatment, and awareness of life-threatening reactions.

INTRODUCTION

Allergy to tree nuts, peanut, and seeds represents a potentially life-threatening condition, and its prevalence has been rising over the past two decades, possibly as a result of its increasing consumption (1,2).

In Europe, tree nuts, peanuts, and seeds are classified as priority food allergens (3,4). Seed storage proteins (SSP) are the most relevant allergens in seeds, and consist of a heterogeneous group of proteins belonging to two different superfamilies; Cupins and Prolamins. They are often designated according to their sedimentation rate: 7S (Vicilins) and 11S (Legumins) Globulins belong to the Cupin superfamily, and 2S Albumins belong to Prolamins (5,6,7). The major allergens and primary sensitizers in legumes, tree nuts and other seeds are contained in these families (2).

These allergens are extremely heat-stable and resistant to digestive enzymes, when compared to Bet v 1 homologs and profilins (5). For this reason, allergic symptoms triggered by IgE binding to SSPs are usually severe, however, it can range from mild reactions (like oral pruritus) to life threatening anaphylaxis (8).

The immunological and biological background for the sensitization to SSPs is not completely understood and significant geographical differences are evident (9, 10)

Ara h 1 (7S Globulins), Ara h 2 (2S Albumins), Ara h 3 (11 S Globulins) are considered major peanut allergens (7). Literature has demonstrated that individuals with confirmed peanut allergy exhibit sensitization predominantly to the 2S Albumin Ara h 2, with lower frequencies of sensitization observed to the 7S Globulin (Ara h 1) and the 11S Globulin (Ara h 3) (4,7).

Cor a 9, from the 11S Globulin family, and Cor a 14, a 2S Albumin, associate with severe hazelnut allergies (11). Ber e 1 is another 2S Albumin, the major allergen of Brazil Nut⁴. 2S Albumins are also prominent allergens in cashew nut (Ana o 3) and walnut (Jug r 1) (12,13).

In a European study it was shown that patients polysensitized to the different storage proteins had a more severe disease than those monosensitized to the 2S albumin component (6,14). Recent studies suggest that sensitization to 2S Albumins, including hazelnut Cor a 14, may be associated with increased severity of allergic reactions (4,11).

Clinically significant cross-reactivity is frequently observed among proteins within the same family. Recent findings, however, show that IgE cross-reactivity can also occur between proteins from different families of SSP (4,15). Sensitization to Cor a 14 (2S Albumins) was highly associated with sensitization to Cor a 9 (11S Albumins) (15). Strong cross-reactivity has been shown for cashew and pistachio, for hazelnut and walnut and for walnut and pecan nut. Cashew nut Ana o 3 and walnut Jug r 1 are considered to be the primary sensitizers (4,16).

The aim of this study was to characterize the molecular and clinical profile of tree nuts, peanut and/or seeds allergic patients sensitized to SSPs, contributing with novel insights into this topic in Southern European patients.

METHODS

Design

A retrospective, observational, descriptive and inferential study was performed in our Allergy and Clinical Immunology department.

Patients and data collection

All patients with a clinical history suggestive of tree nut/seed/peanut allergy underwent an allergological evaluation, initially including skin prick testing and/or measurement of serum specific IgE. Subsequently, patients with sensitization to storage protein components were identified through molecular diagnostics using ImmunoCAP™ ISAC, between 2016 and 2024. Tested SSPs were rAra h 1, rAra h 2, rAra h 3 and rAra h 6 for peanut; nGly m 5, nGly m 6 for soy; nCor a 9, rCor a 14 for hazelnut; rAna o 2, rAna o 3 for cashew nut; rJug r 1 for walnut; rBer e 1 for brazil nut; nSes i 1 for sesame seed and nFag e 2 for buckwheat. Additionally, co-sensitization to non-specific lipid transfer proteins (nsLTPs) was identified through the ISAC microarray, namely to Pru p 3, Jug r 3, and Ara h 9.

The cut-off value for positive determinations was >0.3 ISU E as recommended by the manufacturer.

The severity of the reactions was assessed both quantitatively using the oFASS-5 (table I) scale and qualitatively using the oFASS-3 scale (17).

Details of the elicitor of the most severe reaction (referred to as the 'index reaction') and its severity grade were obtained, as well as information on reactions to or tolerance of other tree nuts, seeds, and peanuts.

Ethics

This study was conducted in accordance with the ethical standards established in the Declaration of Helsinki of 1946. The institutional ethics committee approved the study.

Data analysis

Data analysis was performed using *Statistical Package for Social Sciences* (SPSS) version 28®. Descriptive statistics were used to characterize the sample. Categorical variables were described as absolute and relative frequencies. For variables with normal distribution, we present mean and standard deviation, and for variables without normal distribution, median [minimum and maximum, interquartile range (IQR)]. The χ^2 test was used to measure the correlation between categorical variables. p values below 0,05 indicate statistical significance.

RESULTS

A total of sixty-six patients were included, with a median age of 10 years (range from 1 to 67, IQR:15), 73 % under 18 years old (yo) and 64% were males.

Clinical and demographic characteristics are described in table II.

Symptoms are detailed in Table I, classified according to the oFASS-5 severity grading system (17).

Nineteen patients (29%) reported allergic reactions to more than one type of tree nut, corresponding to an average of 1,4 nuts per patient ($\pm 0,7$). The most frequent co-sensitization pattern involved walnut and hazelnut (32%), with a statistically significant association ($p=0,05$) and an odds ratio (OD) of 12. This subgroup was predominantly sensitized to Juglans regia (n=11), Corylus avellana (n=8), and Anacardium occidentale (n=7). Additionally, more than half of the cohort (n=40, 61%) tolerated other tree nuts distinct from the ones triggering their reaction. The most commonly tolerated nuts in this sample were peanut (n=30, 46%), almond (n=29, 44%), and hazelnut (n=23, 35%). All patients who tolerated pistachio (n=9) also tolerated cashew ($p < 0,01$). Peanut tolerance was significantly associated with tolerance to other tree nuts, namely pistachio (n=9, $p < 0,01$; OR = 19), almond (n=20, $p < 0,01$; OR = 9), hazelnut (n=17, $p < 0,01$; OR = 5), and cashew (n=12, $p = 0,01$; OR = 6). No more associations were found between other nuts. In contrast, after the allergy diagnosis, 38 patients (58%), single-handedly stopped consuming peanut and/or tree nuts, even without previous reaction to that specific food.

Walnut induced allergic reactions (regardless of their severity) in 27 patients (41%), followed by hazelnut and peanut, each with a frequency of 14 patients (21%), and cashew in 10 patients (15%). Symptoms related to almond, chestnut and pine nut were each reported by four patients. Pistachio and buckwheat were each implicated as the culprit food in two patients reporting allergic symptoms. Three patients reported

symptoms with soy. Five patients (all adults) experienced reactions to seeds: one reacted to sesame and sunflower seeds and four to sesame seeds. When analyzing the culprit foods based on the most severe reaction for each patient (the index reaction), walnut was the most prominent (35%), followed by peanut (18%), and cashew and hazelnut, both with 14%. Dividing the sample into two groups, individuals under 18 years of age and those aged 18 yo or older, we observed differences regarding the foods responsible for the index reaction. In the first group (<18 years), the most frequently involved nut was walnut (40%), followed by peanut (21%). In the second group (≥ 18 years), cashew was the most common (28%), followed by walnut (22%). These data are shown in Figure 1.

The distribution of clinical severity related to the most common culprit foods in the index reaction is depicted in table III. It is noteworthy that all index reactions attributed to seeds were severe (oFASS grades 4-5).

Molecular sensitization patterns are depicted in figure 2. The most frequent sensitizer in the pediatric group was Jug r 1 (n=30), while in the adult group it was Ana o 3 (n=6), both followed by Cor a 9 across age groups. On average, patients were sensitized to 2,3 SSP ($\pm 1,6$). Thirty patients (46%) were monosensitized—24 of them with a clinical history of reaction to a single tree nut and six reporting reactions to more than one nut. Among polysensitized patients (n = 36), 13 experienced reactions to multiple nuts, while 23 reported symptoms with only one nut. Of these 23 patients, 8 tolerated other nuts and 15 avoided all other nuts. The number of SSP sensitizations was significantly higher in patients under 18 years old [2 (range 1 to 7, IQR: 2) versus 1 (range 1 to 4, IQR: 1)], with statistical significance (p = 0.03).

Walnut allergy (n=27) was associated with sensitization to Jug r 1 (p<0,01); among those sensitized to Jug r 1 (n=32), one patient tolerated walnut, 23 reported clinical reactions to it, while the remaining individuals do not regularly consume walnut, and

therefore no reactions or tolerance have been reported in these cases. Four patients had confirmed walnut allergy despite the absence of sensitization to Jug r 1. In all four cases, the index reactions were triggered by other foods, including cashew, seeds, and hazelnut. Their SPP sensitization profiles revealed reactivity to Cor a 9 (n=3), Gly m 6 (n=2), Ara h 3 (n=1), and Ses i 1 (n=1).

Peanut allergy (n=14) was associated with sensitization to Ara h 1 (n=4; $p<0,01$), Ara h 2 (n=10; $p=0,03$), and Ara h 6 (n=8; $p<0,01$). Molecular sensitization profile to *Arachis hypogaea* in patients with symptoms with peanut is represented in figure 3. There are two patients who reported symptoms with peanut without sensitization to Ara h 1, Ara h 2 or Ara h 6. These patients were sensitized to Ana o 3 (n=1), Jug r 1 (n=1), Cor a 14 (n=1) and Cor a 9 (n=1). The fruit that triggered the reactions in both cases was cashew. All patients sensitized to Ara h 2 and Ara h 6 did not eat peanut, but one patient sensitized to Ara h 1 tolerated it.

Hazelnut allergy (n=14) was associated with Cor a 14 (n=6; $p<0,01$). Among the eight patients with clinical reactions to hazelnut and no sensitization to Cor a 14, two exhibited sensitizations to Cor a 9. Sensitization to Cor a 9 was not significantly associated with hazelnut allergy (n=5; $p = 0,09$). Among the 19 patients sensitized to Cor a 9, five reported symptoms upon hazelnut consumption, two consumed hazelnuts regularly without any symptoms, and the remaining 12 patients did not include hazelnuts in their diets. Molecular sensitization profile to *Corylus avellana* in patients with symptoms with hazelnut is represented in figure 3. Six patients reacted to hazelnut without sensitization to Cor a 9 or Cor a 14 and the foods implicated in the index reaction were hazelnut (n=3), seeds (n=2), and cashew (n=1). The observed sensitization pattern was Ara h 2 (n=1); Ara h 6 (n=1); Jug r 1 (n=3); Ana o 3 (n=2); Ses i 1 (n=1); and Gly m 6 (n=2).

Cashew allergy (n=10) was associated with sensitization to Ana o 3 ($p < 0,01$). Among the 19 patients sensitized to Ana o 3, one tolerated cashew (confirmed by an oral food challenge), nine experienced symptoms upon cashew ingestion ($p < 0,01$), and the remaining ten did not consume cashew. Of the patients with symptoms with cashew, none were co-sensitized to Ana o 3 and Ana o 2.

Four patients in our cohort (6%) were sensitized to Ana o 2; one reported symptoms with cashew, and three avoided its consumption. This sensitization was associated with clinical symptoms upon almond ingestion (2 out of 4 patients, $p = 0,02$).

Three patients reporting symptoms following **pistachio** exposure were sensitized to Ana o 2 and Ana o 3; two exhibited clinical reactivity to cashew, while the other did not routinely consume it.

Two patients experienced symptoms with **buckwheat**, both of whom were sensitized to Fag e 2 ($p = 0,02$).

Among the three patients reporting **soy**-related symptoms, two were sensitized to Gly m 6 and one to Gly m 5; none exhibited co-sensitization to both allergens.

The most frequent sensitization profiles in patients allergic to **seeds** (n=7) were Ses i 1 (n = 2, 29%) and Gly m 6 (n = 2, 29%).

Sensitization to **2S Albumins** was present in 58 patients (88%) and 38 patients were exclusively sensitized to them. In this group, the most frequent co-sensitizations were between Jug r 1 and Ana o 3 (9 patients), Ara h 6 and Ara h 2 (9 patients), Jug r 1 and Cor a 14 (6 patients), Jug r 1 and Ara h 2 (5 patients), and Jug r 1 and Ara h 6 (4 patients). However, a significant association was found only between Ara h 6 and Ara h 2 ($p < 0,01$). Sensitization to **11 S globulins** was present in 24 patients (36%). Monosensitization to 11 S globulins was found in five patients. Regarding co-sensitizations within this group, they were found between Gly m 6 and Cor a 9 (9 patients,

$p < 0,01$); Ana o 2 and Cor a 9 (4 patients, $p < 0,01$); Gly m 6 and Ana o 2 (4 patients, $p < 0,01$). All patients sensitized to Ana o 2 were sensitized to Gly m 6 and Cor a 9. Sensitization to **7S globulins** occurred in 16 patients (23%). No statistically significant association was observed between the molecular allergens in this group. Regarding sensitizations between different groups, there were statistically significant associations between co-sensitization to 2S albumins and 11S globulins, namely between Cor a 9 and Cor a 14 ($p = 0,03$). Associations between sensitization to 2S albumins and 7S globulins groups were found between Ara h 2 and Ara h 1 ($p = 0,04$). No association was found between sensitization to 7S and 11S globulins. Four patients were co-sensitized to the three groups a and the severity of the index reaction in all these patients was grade 4 or 5.

Based on the oFASS-3 severity scale, the index reaction was classified as mild in eight patients (12%), moderate in 24 (36%), and severe in 34 (52%). Thirty-four patients (52%) reported anaphylaxis (grade 4-5 by oFASS-5), 72% in adult patients and 44% in patients under 18 yo. Co-sensitization between SSPs and nsLTPs (Pru p 3, Jug r 3, and Ara h 9) was associated with the occurrence of anaphylaxis ($p = 0,05$; $OD = 8$), with 7 out of 8 co-sensitized patients (88%) experiencing anaphylaxis. Furthermore, when evaluating reaction severity using the oFASS-5 scale, a statistically significant difference was observed between the co-sensitized and non-co-sensitized groups ($p = 0,01$), with the co-sensitized group showing a higher median severity score [4,5 (range 2 to 5, IQR:1) vs. 3 (range 1 to 5, IQR:2)]. Sensitization to Cor a 14 showed a statistically significant association with grade 5 reaction severity ($p = 0,02$), with 40% of patients sensitized to Cor a 14 experiencing grade 5 reactions. No associations were found with other SPPs. No association was found between the severity of reactions and sensitization to one or multiple SSPs. Age and sex were not associated with the severity of the reactions.

DISCUSSION AND CONCLUSIONS

Approximately one-third of the cohort reported allergic reactions to more than one type of tree nut, with walnut and hazelnut being the most frequently co-reported pair. Interestingly, a substantial proportion of patients (61%) tolerated other tree nuts not implicated in their reactions, with peanut, almond, and hazelnut being the most frequently tolerated. In this study, tolerance to cashew was associated with tolerance to pistachio. These insights have important implications for both diagnosis and treatment. Similar results were reported in the *NutCRACKER* study, where the researchers observed that while a majority of patients allergic to cashews are also allergic to pistachios, nearly all patients allergic to pistachios were also allergic to cashews (16). The authors appropriately point out in their discussion that the grouping of these nut types could have important implications for cross-tolerance, likely due to the presence of related proteins (16). In this cohort, peanut tolerance appeared to be associated with tolerance to other tree nuts, which may have clinical and therapeutic implications; however, further studies are needed to confirm this observation. Despite this, more than half of the cohort reported voluntary avoidance of peanut and/or tree nuts after diagnosis. This behavior likely reflects widespread anxiety surrounding nut allergies among both patients and healthcare professionals. This concern is further exacerbated by the limited and sometimes conflicting data on cross-reactivity between different nuts and seeds, which makes it challenging to provide clear guidance on which foods should be avoided. Traditionally, the medical community has tended to favor broad avoidance strategies, contributing to overly restrictive practices that may not always be clinically justified. These factors highlight the urgent need for targeted education, improved communication regarding real versus perceived risks of cross-reactivity, and structured dietary counseling to help minimize unnecessary dietary restrictions and prevent potential nutritional deficiencies.

It has been reported in the literature that walnut is one of the most common inductors of allergy in Southern Europe, while peanut is the most common in the United Kingdom (10). In concordance, in this study, walnut was the most frequently implicated food; however, regarding the food responsible for the most severe reaction, cashew was the most implicated in individuals over 18 yo, whereas walnut was the most frequent in those under 18 yo. Allergy to seeds was minimal, with no symptoms reported in children, consistent with findings from other studies (4). However, all of these patients experienced severe reactions (grade 4–5 on the oFASS-5 scale), as reported in previous studies, particularly, with sesame seeds (10).

In this study, the most prevalent allergens were 2S albumins, followed by 11S globulins and 7S globulins, as reported in the literature (4,18). Jug r 1, followed by Cor a 9, were the most frequent allergens in the general sample. However, in adult patients, Ana o 3 was the most frequent sensitizer.

Our study revealed a higher number of sensitizations to SSPs in patients under 18 years. The authors postulate that these findings may be explained by recent changes in the consumption patterns of tree nuts, peanuts, and seeds. Specifically, there has been a worldwide decrease in the consumption of these nuts in baked goods or directly from their shells, and an increase in the consumption of unshelled and packaged products (19). This shift may lead to greater exposure to food particles in the air at home, school, and the workplace. Research indicates that early cutaneous allergen exposure may predispose individuals to food sensitizations (20).

IgE to SSP has generally a high predictive value to diagnose an allergy to the respective food, and studies have confirmed its higher diagnostic value when compared to whole extracts IgE, particularly in children (4,7,21). In this research, Jug r 1 appears to be a good marker for walnut allergy, as previous data has shown. Ana o 3 was found to

be predictive of cashew nut allergy, in concordance with previous studies (9,22). It has been reported that IgE to hazelnut Cor a 9 and 14 are highly predictive for hazelnut allergy, and both hazelnut allergens are associated with severe reactions (9,22). In our population, Cor a 14 appears to be a good marker of hazelnut allergy and it was associated with grade 5 reaction severity. Regarding peanut allergy, our data showed clinical relevance of Ara h 1, 2, and 6. In previous studies, IgE to Ara h 2 has a better diagnostic accuracy than IgE to peanut extract, and IgE to Ara h 1, 3 and 6 appear less useful in the diagnosis of peanut allergy than Ara h 2 (7,22).

In our sample, some patients sensitized to SSPs, namely Jug r 1, Ara h 1, and Ana o 3, tolerate the consumption of the respective tree nuts. Therefore, in the presence of SSP sensitization, it is essential to individually assess the possibility of tolerance to avoid unnecessary dietary restrictions and improve patients' quality of life.

In this study, associations between proteins from different groups were found between proteins from the same allergenic sources (Cor a 9 and Cor a 14 from hazelnut and Ara h 1 and Ara h 2 from peanut) in concordance with recent evidence that has shown that IgE cross-reactivity also occurs between members of different protein families (4,15).

The observation that all patients sensitized to Ana o 2 were also co-sensitized to Cor a 9 and Gly m 6 led the authors to hypothesize that Ana o 2 may act as a secondary or later-stage sensitizer, rather than an initial sensitizer. This pattern implies that Cor a 9 and Gly m 6 could be the primary allergens triggering initial sensitization, with Ana o 2 emerging subsequently, possibly due to cross-reactivity or epitope spreading mechanisms. To confirm this hypothesis, a longitudinal study would be necessary to track the sequence of sensitization events in patients exposed to these allergens. Such an approach could help clarify whether sensitization to Ana o 2 indeed tends to occur after prior sensitization to Cor a 9 and Gly m 6.

Similar to other studies, the severity of reactions showed no association with the sex or age of the patients (10), but in our patients, sensitization to multiple SSPs did not associate with the severity of reactions, contrary to previous data (6). Co-sensitization between nLTPs and SSPs was associated with increased severity of allergic reactions.

This study's retrospective design and small sample size from a single clinic limit the generalizability of its findings on sensitization and allergy severity, especially given potential regional differences in allergen exposure. Furthermore, the study does not control for confounding factors like genetics or environmental exposure, and its severity grading system may not capture all the nuances of clinical reactions. These limitations highlight the need for larger, prospective studies and broader population samples to strengthen the findings.

However, this study represents a significant contribution to the understanding of tree nut, peanut, and seed allergies in Portugal, a region with distinct dietary habits and allergenic patterns compared to Northern Europe. By addressing both clinical and molecular aspects, this research bridges a critical knowledge gap and lays the groundwork for improved diagnostic and therapeutic approaches tailored to the Portuguese population.

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AUTHOR CONTRIBUTIONS:

ACC: Conceptualization, data curation, methodology, visualization, formal analysis, writing – original draft, writing – review & editing.

ML and MA: Data curation, methodology and visualization

SC: Conceptualization, writing – review & editing.

JQG and ARF: Conceptualization, methodology, validation, writing – review & editing,

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CONFLICT OF INTEREST

The authors declare that no conflicts of interest exist.

REFERENCES

1. Eigenmann PA, Lack G, Mazon A, Nieto A, Haddad D, Brough HA, et al. Managing Nut Allergy: A Remaining Clinical Challenge. *J Allergy Clin Immunol Pract.* 2017;5(2):296-300. doi: 10.1016/j.jaip.2016.08.014.
2. Weinberger T, Sicherer S. Current perspectives on tree nut allergy: a review. *J Asthma Allergy.* 2018;11:41-51. doi: 10.2147/JAA.S141636.
3. Ibáñez-Sandin MD, Rodríguez Del Río P, Alvarado MI, García BE, Garriga-Baraut T, Reche Frutos M, et al. Onset of Nut Allergy in a Pediatric Cohort: Clinical and Molecular Patterns in the AFRUSEN Study. *J Investig Allergol Clin Immunol.* 2022;32(4):270-281. doi:10.18176/jiaci.0696.
4. Dramburg S, Hilger C, Santos AF, de Las Vecillas L, Aalberse RC, Acevedo N, et al. EAACI Molecular Allergology User's Guide 2.0. *Pediatr Allergy Immunol.* 2023 Mar;34 Suppl 28:e13854. doi: 10.1111/pai.13854.
5. Rasheed F, Markgren J, Hedenqvist M, Johansson E. Modeling to Understand Plant Protein Structure-Function Relationships-Implications for Seed Storage Proteins. *Molecules.* 2020 Feb 17;25(4):873. doi: 10.3390/molecules25040873.
6. Hed J. Cross-reactivity in plant food allergy-Clinical impact of Component Resolved Diagnostics (CRD). Phadia AB, 2008. Uppsala (Sweden).
7. Klemans RJ, van Os-Medendorp H, Blankestijn M, Bruijnzeel-Koomen CA, Knol EF, Knulst AC. Diagnostic accuracy of specific IgE to components in diagnosing peanut allergy: a systematic review. *Clin Exp Allergy.* 2015;45(4):720-730, doi: 10.1111/cea.12412.
8. McWilliam V, Koplin J, Lodge C, Tang M, Dharmage S, Allen K. The prevalence of tree nut allergy: a systematic review. *Curr Allergy Asthma Rep* 2015;15:54.
9. Burney PG, Potts J, Kummeling I, Mills EN, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitization in European adults. *Allergy* 2014;69:365-71.
10. Brough HA, Caubet J-C, Mazon A, Haddad D, Bergmann MM, Wassenberg J, et al. Defining challenge-proven coexistent nut and sesame seed allergy: A

- prospective multicenter European study. *J Allergy Clin Immunol.* 2020;145(4):1231-9. doi: 10.1016/j.jaci.2019.09.036.
11. Masthoff LJ, Mattsson L, Zuidmeer-Jongejan L, Lidholm J, Andersson K, Akkerdaas JH, et al. Sensitisation to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. *J Allergy Clin Immunol.* 2013;132(2):393-399. doi: 10.1016/j.jaci.2013.02.024.
 12. Lange L, Lasota L, Finger A, Vlajnic D, Büsing S, Meister J, et al. Ana o 3-specific IgE is a good predictor for clinically relevant cashew allergy in children. *Allergy.* 2017;72(4):598-603,doi: 10.1111/all.13050, PMID: 27644013.
 13. Sato S, Yamamoto M, Yanagida N, Ito K, Ohya Y, Imai T, et al. Jug r 1 sensitisation is important in walnut-allergic children and youth. *J Allergy Clin Immunol Pract.* 2017;5(6):1784- 1786 e1781, doi: 10.1016/j.jaip.2017.04.025, PMID: 28552380.
 14. Bublin M, Breiteneder H. Cross-reactivity of peanut allergens. *Curr Allergy Asthma Rep.* 2014 Apr;14(4):426. doi: 10.1007/s11882-014-0426-8. PMID: 24554241; PMCID: PMC3962743
 15. Lyons SA, Datema MR, Le TM, Asero R, Barreales L, Belohlavkova S, et al. Walnut Allergy Across Europe: Distribution of Allergen Sensitisation Patterns and Prediction of Severity. *J Allergy Clin Immunol Pract.* 2021;9(1):225-235.e10. doi:10.1016/j.jaip.2020.08.051.
 16. Elizur A, Appel MY, Nachshon L, Levy MB, Epstein-Rigbi N, Golobov K, et al. NUT Co Reactivity - ACquiring Knowledge for Elimination Recommendations (NUT CRACKER) study. *Allergy.* 2018;73(3):593-601. doi:10.1111/all.13353.
 17. Fernández-Rivas M, Gómez García I, Gonzalo-Fernández A, Fuentes Ferrer M, Dölle-Bierke S, Marco-Martín G, et al. Development and validation of the food allergy severity score. *Allergy.* 2022 May;77(5):1545-1558. doi: 10.1111/all.15165.
 18. Packi K, Matysiak J, Matuszewska E, Bręborowicz A, Matysiak J. Changes in Serum Protein-Peptide Patterns in Atopic Children Allergic to Plant Storage Proteins. *Int J Mol Sci.* 2023;24(2):1804. doi:10.3390/ijms24021804.
 19. Grand View Research. Packaged Nuts & Seeds Market Size, Share & Trends Analysis Report By Product (Nuts, Seeds), By Distribution Channel, By Region, And Segment Forecasts, 2024 - 2030 [Internet]. San Francisco: Grand View

Research; 2024. Available from: <https://www.grandviewresearch.com/industry-analysis/packaged-nuts-seeds-market#>

20. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *J Allergy Clin Immunol.* 2018;141(1):41-58. doi:10.1016/j.jaci.2017.11.003.
21. Blankestijn MA, Blom WM, Otten HG, Baumert JL, Taylor SL, Bruijnzeel-Koomen CA, et al. Specific IgE to Jug r 1 has no additional value compared with extractbased testing in diagnosing walnut allergy in adults. *J Allergy Clin Immunol.* 2017;139(2):688-690 e684, doi: 10.1016/j.jaci.2016.07.026, PMID: 27597723 .
22. Geiselhart S, Hoffmann-Sommergruber K, Bublin M. Tree nut allergens. *Mol Immunol.* 2018;100:71-81. doi: 10.1016/j.molimm.2018.03.011

Table I. Classification of the most severe (index) reaction and number of patients according to oFASS-5 classification.

oFASS-5	Oral cavity	Skin / Nose / Eye / Digestive / Uterus	Larynx / Bronchi	Cardiovascular / Nervous system	n (%)
Grade 1	Yes	No	No	No	8 (12)
Grade 2	Yes / No	1 system	No	No	13 (20)
Grade 3	Yes / No	>1 system	No	No	11 (17)
Grade 4	Yes / No	Yes / No	1 or both	No	25 (38)
Grade 5	Yes / No	Yes / No	Yes / No	1 or both	9 (14)

Table II. Demographic and clinical characteristics of patients.

n, total	66
Sex, male n (%)	42 (64)
Age in years median (min-max, IQR)	10 (1-67, 15)
Patients <18 yo n (%)	48 (73)
Comorbidities n (%)	
Asthma	25 (38)
Rhinitis	43 (65)
Atopic dermatitis	18 (27)
Other food allergy	28 (42)
Co-sensitization SPPs-nLTPs	8 (12)

Table III. Clinical severity distribution of the most common culprit foods in index reaction.

Severity grade oFASS-3	Walnut, n (%)	Peanut, n (%)	Hazelnut, n (%)	Cashew, n (%)	Seeds, n (%)
1 (mild)	2 (9)	1 (8)	2 (20)	1 (11)	0
2-3 (moderate)	8 (35)	6 (50)	4 (40)	4 (44)	0
4-5 (severe)	13 (57)	5 (42)	4 (40)	4 (44)	5 (100)

Figure 1. a) Number of allergic reactions by culprit food, regardless of severity. b) Number of index reactions (more severe reaction) by culprit food.

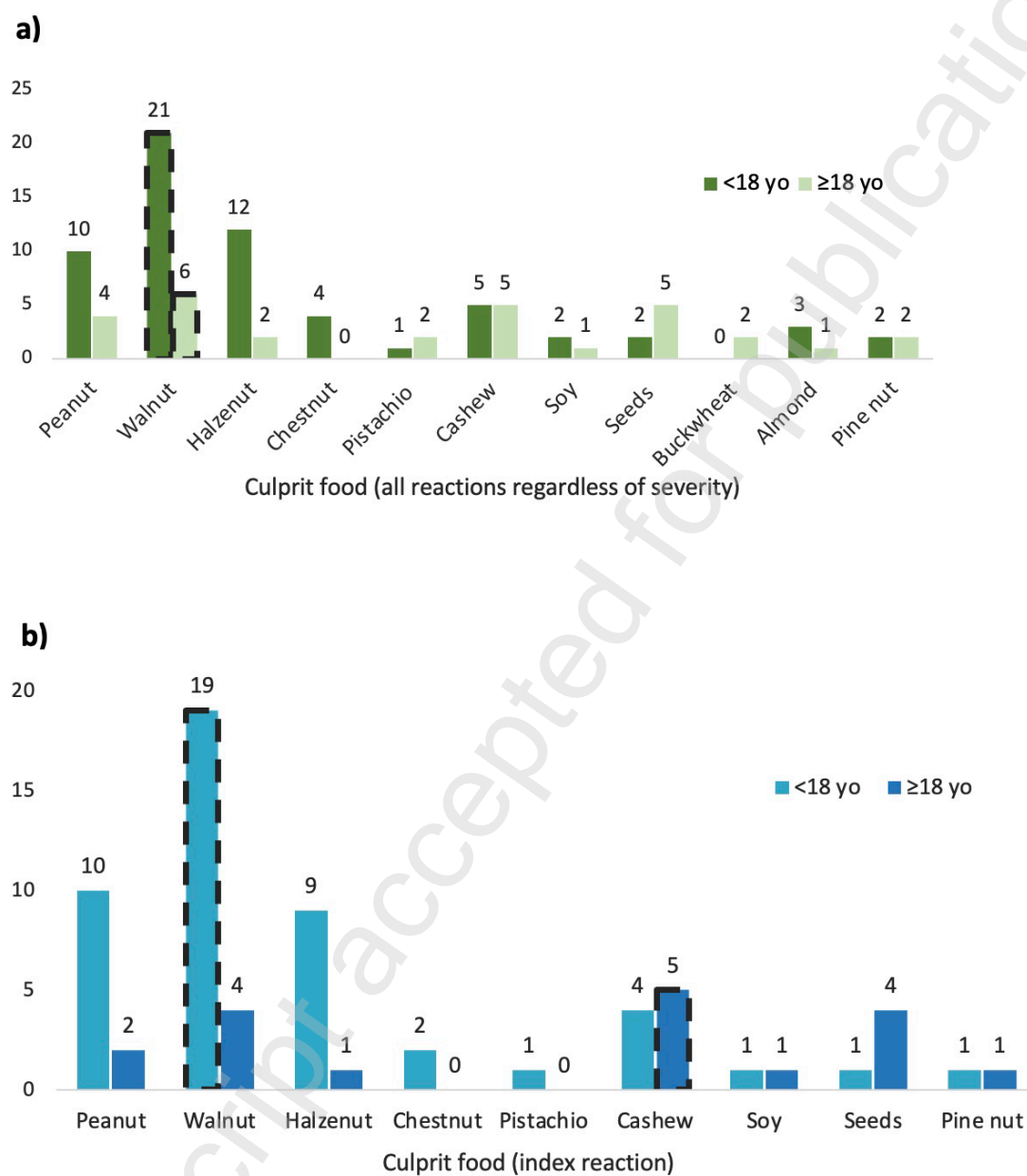


Figure 2. Molecular profile of SSPs sensitization

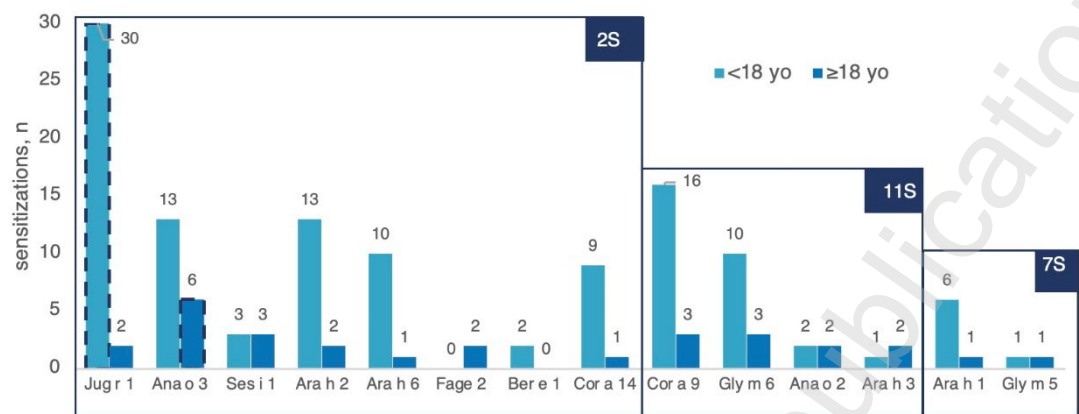


Figure 3. Molecular sensitization profile to *Arachis hypogaea* (Ara h) and *Corylus avellana* (Cor a) in patients with symptoms with Peanut and Hazelnut, respectively.

