A taste of food allergy

Digesting the evidence on patient characterization and management strategies

E.J.J. Kallen

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Digesting the evidence on patient characterization and management strategies

Emily Kallen

A taste of food allergy: Digesting the evidence on patient characterization and management strategies
PhD thesis, Utrecht University, the Netherlands

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A taste of food allergy

Digesting the evidence on patient characterization and management strategies

Inzicht in voedselallergie

Het verteren van het bewijs van patiëntkarakterisering en behandelstrategieën
(met een samenvatting in het Nederlands)

Proefschrift

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1

General introduction

Background on food allergy

Food allergy is a major public health issue with a prevalence of self-reported food allergy in adults across Europe ranging from 2-37% for any food.[1] However, the true prevalence of food allergy confirmed by oral food challenge (OFC) is between 0.2-4.1%.[2,3]

Theoretically, any food can cause a food allergic reaction. However, most studies have concentrated on the prevalence of allergy to cow's milk, hen's egg, wheat, soy, peanut, tree nut, fish, and shellfish, as these foods are thought to cause the majority of allergic reactions, especially in children.[4,5] The EuroPrevall study highlighted the relevance of other foods by investigating 24 foods.[2] Nevertheless, these foods represent only a minority of all foods causing food allergy. Moreover, globalization, climate change and a growing world population are driving changes in dietary habits, including a shift towards a more plant-based diet and the introduction of novel foods.[6–8] Consequently, it is expected that allergies to plant-based foods and novel foods will become more prevalent, underscoring the importance of investigating the full spectrum of foods in prevalence studies.

Food allergy can cause a wide variety of symptoms, including oropharyngeal (e.g. itch), upper respiratory (e.g. rhinitis), skin (e.g. urticaria), gastro-intestinal (e.g. vomiting), lower respiratory (e.g. dyspnea), and cardiovascular symptoms (e.g. drop in blood pressure).[9] Multiple factors may influence the severity of a food allergic reaction. For peanut[10], walnut[11], and hazelnut[12], a specific combination of clinical characteristics and immunologic markers is better in predicting a severe food allergy than clinical characteristics alone. However, less is known about predictors of severity for other foods.

The health-related quality of life (HRQL) of food allergic patients is considerably impaired.[13–16] They often fear allergic reactions and are continuously confronted with dietary and social restrictions in their daily life. Disease specific quality of life, as measured with the Food Allergy Quality of Life Questionnaires (FAQLQ) is an important patient-reported outcome measure assessing the impact of food allergy on allergen avoidance and dietary restrictions, emotional impact, risk of accidental exposure, and food allergy related health.[17] Various factors may influence the HRQL, including a higher number of food allergies[14], the type of food involved[16], cross-cultural differences such as eating habits[13], and the age of onset[15]. Given the importance of this outcome for patients and the fact that it is not usually captured by traditional clinical outcomes, we recommend that HRQL is routinely incorporated into food allergy research and clinical care.

Diagnostic challenges in food allergy

Food allergy is defined as an adverse reaction to food caused by an abnormal immune response.[18] Sensitization refers to the first step in this process, where the immune system recognize a food protein as foreign and produces specific IgE antibodies against it.[19] Not everyone who is sensitized will develop clinical allergic symptoms. Therefore, clinical his-

tory taking by an experienced food allergy specialist is the cornerstone of food allergy diagnosis.[18] Following an allergy-focused history, evidence of allergen specific IgE (sIgE) to the culprit food can support the diagnosis of food allergy.[18] sIgE may be measured through one or more of the following tests: 1) skin prick tests (SPT) using allergen extracts, 2) prick to prick tests (PTP) using fresh foods, 3) sIgE to allergen extracts in blood, and 4) sIgE to allergen components (i.e. component-resolved diagnostics (CRD) in blood.

CRD enables the identification of sensitization profiles at the molecular level.[20] The most common and clinically relevant plant food allergens include pathogenesis-related (PR)-10 proteins, non-specific lipid transfer proteins (LTP), and seed storage (SS) proteins.[21] The PR-10 proteins are major cross-reactive allergens in *Rosaceae* fruits, tree nuts, and legumes. Sensitization is typically initiated by exposure to birch pollen, with the birch pollen major allergen Bet v 1 acting as the primary sensitizer.[22] Based on IgE cross-reactivity, PR-10 homologues in food are subsequently recognized. The PR-10 proteins are found in both the pulp and skin/peel of these foods.[21] In general, they are structurally labile proteins being affected by processing such as heat treatment and by low pH and proteolytic enzymes of the gastro-intestinal tract. They typically induce mild, local reactions. However, more severe symptoms are also reported. Non-specific lipid transfer proteins (LTPs) are major allergens for instance in *Rosaceae* fruits, tree nuts, citrus fruit, and kiwi. LTP sensitization typically originates from exposure to food, with peach LTP, Pru p 3, considered the main primary sensitizer.[23,24] These proteins are predominantly located in the peel.[21] In contrast to PR-10 proteins, LTPs are quite stable and resistant to heat treatment and gastro-intestinal digestion. Therefore, LTPs are more often associated with more severe, systemic reactions. Seed storage proteins (SSP) are found in tree nuts, seeds, and legumes. They are also known for their resistance to digestion and heat. Among these, 2S albumins are the most resistant family members and may be related to more severe allergic reactions.

Sensitization to PR-10 proteins is mostly observed in Northern and Central Europe (birch pollen endemic region), while sensitization to LTP is more prevalent in Southern Europe, especially Mediterranean countries. However, a study on walnut allergy showed that the walnut PR-10 Jug r 5 was quite common in Southern Europe and LTP Jug r 3 was not uncommon in Northern and Central Europe.[11] This indicates that geographical sensitization patterns are more heterogeneous than previously assumed, underscoring the need for broader investigations into molecular allergy profiles.

CRD may improve the estimation of the risk of an allergic reaction.[25] Among the various protein families, SSP, particularly the 2S albumins, are known for their high diagnostic value in children with peanut,[26] hazelnut,[27] or cashew allergy[28]. In adults, however, findings are more heterogeneous: the diagnostic value is high for peanut allergy,[29] moderately high for walnut allergy,[30] and only moderate for hazelnut[12,27,31]. The diagnostic value of 2S albumins has not yet been established for cashew allergy in adults, nor for almond allergy

in either adults or children. However, both almond and cashew are the most consumed nuts worldwide and are one of the main allergens causing anaphylaxis.[32,33] This highlights the need to investigate the diagnostic performance of their respective 2S albumins as well.

The oral food challenge (OFC) is the diagnostic gold standard for food allergy.[18] During an OFC, patients are given gradually increasing doses of the potentially allergenic food to determine whether they are allergic, how severe the reaction may be, and at which dose the first symptoms occur (i.e., the threshold).[18] Nevertheless, OFCs are not without risks as anaphylaxis occurs in 2.4% of the challenges.[34] Changes in physiologic parameters (e.g. heart rate and respiratory rate) may precede an allergic reaction. Early detection of these changes by continuous monitoring may provide opportunities for early recognition, intervention or even prevention of a (severe) reaction. However, it is currently unknown whether changes in physiologic parameters can predict an allergic reaction early, and which parameters are most predictive.

Management of food allergy: optimizing labelling and dietary advice

At present, there is no registered therapy for food allergic adults in the Netherlands. Management therefore focuses on dietary advice and emergency treatment depending on the severity of an allergic reaction.[35] Proper dietary management, including avoidance of the culprit food and continued consumption of tolerated safe foods, is essential and is best implemented under the guidance of a specialized dietician.

Avoidance of the culprit food can be supported by accurate precautionary allergen labeling (PAL). The aim of PAL is to inform and protect consumers with a food allergy.[36] At this moment, the following 14 foods (or food groups) and their products are labelled: cereals containing gluten, crustaceans, egg, fish, soybean, milk, nuts, celery, mustard, sesame, sulfur dioxide and sulfite, lupin, and mollusks.[37] As mentioned above, globalization, climate change and a growing world population are driving dietary changes [6–8], which may in turn affect the frequency and severity of the foods causing allergic reactions. Therefore, it is important to monitor emerging food allergies to ensure that the list of labeled allergens is updated in a timely manner, thereby safeguarding consumers.

Processing may alter the allergenic properties of foods.[38] However, for many foods, the impact of different processing methods on allergenic properties is poorly characterized. This is for example the case for soy, which is an attractive protein source and widely used in plant-based foods. In Western countries, soy consumption has increased over the past decade due to the growing popularity of veg(etari)an diets.[39,40] This trend may lead to an increase in soy allergy. Soy-derived products vary in their processing methods, yet the frequency and severity of allergic reactions to these different products remain largely unknown. With increasing consumption of plant-based proteins, it is important to inves-

tigate how processing affects allergenicity. This will enable patients to be informed about what to eat and what to avoid, and therefore receive the best dietary advice.

Birch pollen-related food allergy: patient profiling and improving management

Birch pollen-related food allergy (BPFA) is the most common form of food allergy in Western and Central Europe. It is caused by cross-reactivity between the birch pollen PR-10 protein Bet v 1, the major birch pollen allergen, and homologues in foods.[7] Although exact prevalence data is unknown, studies indicate that approximately 70% of birch pollen-allergic patients report allergic reactions to certain foods.[7]

The foods involved include *Rosaceae* fruits such as apple and peach, tree nuts such as hazelnut and almond, legumes such as soy and peanut, and other vegetables such as celery and carrot[14,21]. Patients often report symptoms to multiple foods with a range of 4-13 foods.[14] In the literature, patients typically report mild oropharyngeal symptoms but more severe symptoms do occur.[14,41] This is mostly related to (raw) tree nuts, *Rosaceae* fruits, and soy milk.[14,41] However, studies investigating symptom severity in BPFA using oral food challenges (OFCs), the diagnostic gold standard, are scarce.

For diagnosing BPFA, OFCs are also the gold standard. However, many foods are implicated, and in hospital challenges are rarely performed.[21] Home-based oral food challenges (HOFC) may provide a practical alternative, especially for patients with a history of mild symptoms. However, no studies have investigated the safety of HOFC for food allergy in general nor for BPFA specifically.

To date, no treatment is available for patients with BPFA. However, given that BPFA is caused by cross-sensitization to birch pollen, birch pollen immunotherapy may also provide therapeutic benefits for concomitant food allergies.[21] Both subcutaneous (SCIT) and sublingual (SLIT) immunotherapy are available for birch pollen allergy, but their effectiveness in alleviating food-related symptoms in BPFA remains uncertain. Most studies were performed on birch pollen-associated apple allergy, as the apple allergen Mal d 1 shares a high sequence identity to Bet v 1, suggesting that birch pollen AIT might be highly effective.[42–48] Nonetheless, results have been inconsistent, and a comprehensive overview comparing these studies is currently lacking, highlighting the need to investigate suitable therapies for BPFA.

Outline of this thesis

This thesis address questions of food allergy by investigating patient characterizations including sensitization patterns, symptom severity and quality of life, and evaluating management strategies such as dietary guidance, home-based oral food challenges, and potential immunotherapy approaches.

In **Chapter 2**, we investigate the frequency and severity of symptoms to all foods reported to cause allergy in a large group of over 1000 adult patients visiting our outpatient allergology clinic, to reveal the most important foods. In **Chapter 3**, we examine geographical variations in sensitization patterns to peach components across Europe and Japan and evaluate predictors of severe peach allergy in these countries. In **Chapter 4 and 5** we analyze, in adults with a suspected almond allergy, the clinical characteristics and diagnostic value of almond extracts (by SPT and IgE) and the diagnostic value of its components. The diagnostic value of cashew extract versus the component 2S albumin Ana o 3 is assessed in **Chapter 6**. In **Chapter 7** we investigate whether physiological parameters continuously measured by a sensor during an OFC can predict an allergic reaction before the onset of objective symptoms. **Chapter 8** presents dietary advice based on the frequency and severity of eight commonly consumed soy products, which vary in their processing and are expected to elicit a wide range of reactions. It also identifies predictors for severe soy allergy. The profile of patients with BPFA and the safety of home-based OFC are explored in **Chapter 9**, while treatment options and effectiveness of birch pollen SLIT in patients with BPFA are presented in **Chapter 10** and **11**, respectively. Finally, the clinical implications and future perspectives of our findings are discussed in **Chapter 12**.

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2

The frequency and severity of 192 foods causing a food allergy in adults

Kallen E.J.J., Welsing P.M.J., Nijenhuis I., Van Ree R., Knulst A.C., Le T.T.M.

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Introduction

So far, studies mainly focused on the frequency and severity of the so-called 'Big Eight' foods, and less is known about other foods.

Objective

To investigate the frequency and severity of allergy to all foods reported to cause food allergic symptoms.

Methods

Adult patients with symptoms within two hours after ingestion of any food were included, to get the best possible estimate of all foods causing food allergy. Sensitization was measured by skin prick test and/or specific IgE for the culprit food(s) when possible.

Results

A total of 1085 patients were included, of whom 66.9% had a probable food allergy (FA) (i.e. reported symptoms with sensitization) and 33.1% only a possible FA (reported symptoms, independent of sensitization). Patients reported symptoms to a total of 192 foods and sufficient sensitization data was available for 45 foods. The ranking in frequency and severity of symptoms was quite comparable between probable and possible FA. Regarding possible FA, fruit was most commonly reported (68.8%), followed by nuts (63.0%), legumes (39.5%), vegetables (34.0%) and seeds or pits (14.4%). Severe symptoms were most frequently reported for seeds or pits (39.8%), fish (39.2%), legumes (34.8%), nuts (31.9%) and crustaceans (31.3%), and least frequently for fruit (16.5%). Nevertheless, in absolute numbers, fruit rank in position 3 (N=125), with especially tropical fruit (e.g. lychee, papaya, jackfruit) causing severe symptoms frequently. Regarding probable FA, severe symptoms were most common for sunflower seeds (80.0%), pine nut (66.7%), Brazil nut (60.0%), cashew nut (57.1%), and pistachio (51.7%)

Conclusion

Food allergies are caused by almost 200 different foods in adults. Although not mandatory labeled, seeds and pits most commonly cause severe symptoms and fruit cause severe symptoms in a large number of patients. This study highlights the importance of food allergies

Abstract

INTRODUCTION

Food allergy (FA) is an important global human health issue, and it imposes a growing societal burden.[1] The estimated lifetime prevalence of FA in Europe has increased from 2.6% in 2000-2012 to 3.5% in 2012-2021[2] and it is expected to increase further in the future[1].

Theoretically, any food can cause a FA, but prevalence studies have mainly focused on the so-called big eight foods, i.e., cow's milk, hen's egg, wheat, soy, peanut, tree nuts, fish, and shellfish and less is known about the prevalence of food allergy for other foods.[2–6] Two studies have shown that foods outside the big eight that were traditionally not considered important are in fact very relevant FA, such as kiwi, peach and apple.[3,7] Due to globalization, climate change and a growing world population, there is change in dietary habits with a shift towards a more plant-based diet.[4–6] Due to this shift, it is expected that allergies to plant foods will increase. Furthermore, the introduction of novel foods can cause new FA. Therefore, studies should not only focus on the big eight, but on the prevalence and severity of food allergies for all foods, especially plant-based foods and novel foods.

Oral food challenge (OFC) remains the gold standard for diagnosing food allergy (FA).[8] However, in clinical practice, it is often not feasible to perform OFCs for all suspected foods in every patient. Sensitization tests support diagnosis, but for many foods, no commercial diagnostic tests are available. Diagnostic certainty can be improved through a detailed clinical history taken by an experienced allergology specialist. Additionally, one of our earlier studies demonstrated that the presence of oral allergy symptoms and allergic rhinitis are among the strongest predictors of probable food allergy.[9] Studies often report data only for a limited number of foods for which both symptoms and sensitization are confirmed (probable FA). However, data on reported symptoms (possible FA) can be relevant as well. The lack of sensitization data for a number of foods reflects the diagnostic gap frequently encountered in clinical practice. In this study, we aimed to combine these categories to provide the best representation of all foods causing symptoms, based on clinical history taken by highly trained food allergy specialists, while incorporating sensitization data whenever available.

The aim of this study was to investigate the prevalence and severity of all foods that were reported to cause a reaction by adult patients at our outpatient allergology clinic.

METHODS

Study population

All consecutive adult patients (≥ 18 years) who were referred to a tertiary center in The Netherlands, the allergology outpatient clinic of the University Medical Centre Utrecht (UMCU), with reported symptoms to food within two hours after ingestion (possible FA) between July 2018 and September 2023 were included retrospectively. Patients were excluded if they reported symptoms after ingestion of a composite meal where the causative food ingredient was unclear, or if they objected to their data being used for research purposes.

This study was approved by the Medical Research Ethics Committee of the UMCU (protocol number 22-983).

Data collection

Clinical questionnaire

Physicians systematically collected clinical information from all patients with a suspected FA during the first outpatient clinic visit, using a standardized questionnaire. This involved: demographics, allergic comorbidities, culprit food(s), reported symptoms, severity, time interval and prescription of emergency medication (antihistamines, prednisone and/or epinephrine auto-injector).

Classification of severity

The validated oFASS-3 score was used to classify the severity; oFASS-3 grade 1 includes isolated oropharyngeal symptoms; grade 2 may include grade 1 symptoms, but at least symptoms of the skin, eye, upper airways and/or digestive system and grade 3 may include grade 1 and/or 2 symptoms, but at least symptoms of the lower airways, cardiovascular and/or nervous system.[10] In this article, grade 1 will be referred to as mild, grade 2 as moderate and grade 3 as a severe reaction.

Specific IgE measurements

Sensitization was measured by skin prick tests (SPT) (ALK, Madrid, Spain), prick-to-prick tests (PTP) and serum specific IgE (sIgE) tests (including component resolved diagnostics [CRD]) (ImmunoCAP, ThermoFisher Scientific, Uppsala, Sweden) for foods for which tests were available. A mean wheal diameter ≥ 3 mm and sIgE levels ≥ 0.35 KU_A/L were considered positive, respectively. The tests performed in each patient was at the discretion of the physician and depended on factors such as the number of reported foods, the severity of the culprit food, availability of commercial tests for the food and the patient's wishes.

Definitions

Possible FA: patients with reported symptoms to food, independent of sensitization.

Probable FA: patients with reported symptoms and matching sensitization of the food.[11] Probable FA was only investigated for foods for which sensitization was measured in at least 10 patients who also reported symptoms to these foods. Although using probable FA data is preferable over possible FA, diagnostic tests for many novel or less commonly consumed foods were unavailable and the diagnostic value of sIgE is not known for less common foods. Our aim was to assess the frequency and severity of **all** foods and excluding patients with possible FA would implicate the loss of a significant portion of valuable real-word data. We investigated whether the severity differed between patients with a possible and probable FA for the foods for which diagnostic tests were available, to see if severity could be generalized from possible FA to probable FA.

Food groups: To compare the frequency and severity of corresponding foods, the individual foods were classified into 14 food groups: 1) fruits, 2) nuts, 3) legumes, 4) vegetables, 5) seeds and pits, 6) dairy, 7) hen's egg, 8) crustaceans, 9) fish, 10) grains, 11) mollusks, 12) spices and herbs, 13) meat, and 14) insects.

Co-allergies: Co-allergies were defined as patients with reported symptoms to one food who also reported symptoms to another food, regardless of their sensitization pattern.

Sensitization patterns in patients with reactions to plant foods

As plant foods are the most common allergenic sources in adults, and as this may become an even greater problem with a shift towards a more plant-based diet, the relationship between the severity of symptoms and the underlying molecular sensitization pattern of plant foods was investigated.

For this purpose, patients who reported symptoms to at least one plant food were divided into the following groups according to their sensitization pattern:

- PR-10 group: sensitization to only PR-10 and not to lipid transfer proteins (LTP) and seed storage proteins (SSP);
- LTP group: sensitization to only LTP and not to PR-10 and SSP;
- SS group: sensitization to only SSP and not to PR-10 and SSP;
- PR-10/LTP group: sensitization to PR-10 and LTP and not to SSP;
- PR-10/SS group: sensitization to PR-10 and SSP and not to LTP;
- LTP/SS group: sensitization to LTP and SSP and not to PR-10;
- PR-10/LTP/SS group: sensitization to PR-10, LTP and SSP

The following components were measured:

- PR-10: Cor a 1.0401 and/or PR-10 mix (Bet v 1, Que a 1, Mal d 1, Jug r 5 and Ara h 8)
- LTP: LTP mix (Pru p 3, Tri a 14, Pha v 3, Lac s 1, Mal d 3, Jug r 3, Art v 3, Cor a 8 and Ara h 9)
- SS: Ara h 2, peanut storage mix (Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 7) Cor a 9, Cor a 14, nut storage protein mix (Ana o 3, Jug r 1, Ber e 1) and/or Tri a 19

IgE measurement for classifying the plant food groups was performed with customized immunoblots (EUROLINE, EUROIMMUN, Lübeck, Germany). Sensitization was assessed according to the standard manufacturer's instructions using the EUROBlotOne. The EUROLINE intensity units (EL) were evaluated using the EUROLineScan software. Results of ≥ EAST class 1 were considered positive.

As the immunoblots were routinely measured in every patient since March 2022, the analyses of the subgroups based on sensitization pattern were performed on patients visiting our clinic from March 2022.

In addition to the beformenentiond classification based on the immunoblot where multiple food mixes were measured, a classification based on ImmunoCAP was made for individual foods. For valid conclusions based on CRD, all allergen families or at least the most important allergen families, must be measured for the culprit food. Therefore, only those foods for which both PR-10 and non-PR-10 components were measured by ImmunoCAP were used.

Statistical analysis

Data was extracted from the hospital's electronic health records. Continuous variables were described as means with standard deviation (SD) when normally distributed and as medians with interquartile range (Q1, Q3) when not normally distributed. Categorical variables were described as frequency and percentages. Patient characteristics were described for the total population.

The highest severity score per patient was defined and described. The frequency of reported symptoms and associated severity of the foods were ranked from most common to least common.

In patients with a plant food allergy, the severity of the different underlying molecular sensitization groups was compared using the Chi-Square trend test ('linear-by-linear' association) to account for the ordinal structure of the severity classification. P values ≤ 0.05 were considered statistically significant.

Analyses were performed using IBM SPSS Statistics version 29.0 and R version 4.2.2.

RESULTS

Clinical characteristics

A total of 1085 adult patients reporting symptoms to 192 foods within 2 hours after ingestion (possible FA) were enrolled. Of these, 70.8% were female. Characteristics of these patients are shown in **Table 1**. The most common atopic comorbidity was allergic rhinitis (AR) which was present in 85.9% of patients. The median number of foods that was reported to cause a reaction within a patient was five, with a maximum of 31. Of all the foods that were reported to cause a reaction per patient, the most severe reaction was mild in 17.6% of the patients, moderate in 40.8% and severe in 41.6%.

As there is likely to be over-reporting if only data on possible FA is taken into account, we also assessed probable FA (reported symptoms + sensitization). Enough sensitization data was available for 45 foods in 945 patients of whom 726 were sensitized, resulting in 76.1% of patients with at least a probable FA (**Table 1**). Data regarding age, sex, atopic co-morbidities, medication, number of foods, highest severity score, and onset of symptoms were comparable between patients with possible and probable FA, supporting the value of data on possible FA.

Table 1. Patient characteristics with reported symptoms to foods within 2 hours after ingestion

	Possible food allergy N = 1085	**Probable food allergy N = 726**
Age in years; mean (SD)	37 (14)	36 (14)
Sex, female; n (%)	768 (70.8)	494 (68.0)
History of atopic comorbidities; n (%)*		
Allergic rhinitis	911 (85.9)	660 (92.4)
Allergic asthma	472 (44.9)	355 (50.1)
Atopic eczema	579 (55.0)	433 (61.0)
Medication; n (%)***		
Antihistamine	973 (93.1)	681 (96.2)
Prednisone	633 (66.5)	463 (72.0)
Adrenaline auto-injector	480 (55.8)	347 (60.3)
Number of foods; median (IQR)	5 (2-8)	6 (3-9)
Highest severity score*; n (%)**	N = 1056	N = 713
Mild	186 (17.6)	116 (16.3)
Moderate	431 (40.8)	207 (37.9)
Severe	439 (41.6)	327 (45.9)
Fastest onset of symptom; n (%)	N = 1085	N = 726

Table 1. Patient characteristics with reported symptoms to foods within 2 hours after ingestion (*continued*)

	Possible food allergy N = 1085	Probable food allergy N = 726
<5 min	906 (83.5)	649 (89.4)
5-30 min	138 (12.7)	66 (9.1)
30-120 min	41 (3.8)	11 (1.5)

*The percentage given in brackets is the percentage of the available information
**Medication could be prescribed for patient their food allergy and/or their co-morbidities
***Mild: isolated oropharyngeal symptoms; moderate: symptoms of the skin, eye, upper airways with/without digestive system +/- mild symptoms; severe: symptoms of the lower airways, cardiovascular with/without nervous system +/- mild/moderate symptoms

Patients with a possible food allergy

Individual foods

Of the patients with a possible FA, apple allergy was most commonly reported with 44.4% (**Table 2**). About a third of the patients reported symptoms to kiwi, hazelnut, walnut or peanut, about a quarter to cherry and a fifth to almond, peach or pear. Almost 80% of the reported symptoms were caused by 30 foods and 20% by the remaining 162 foods, of which 35 foods were only reported once. Although mandatory labelled, symptoms were uncommon for lupine (0.1%), fish (0.1 – 1.9%), crustaceans other than shrimp (0.2 – 1.4%), mustard (1.1%), and celery (2.5%).

When focusing on the frequency of severe symptoms of the individual foods for which at least 15 patients reported symptoms, pine nut, sunflower seed, peanut, cashew nut, and salmon most frequently caused severe symptoms (38.9% - 50.9%)(**Table 2**). However, in absolute numbers, peanut, hazelnut, walnut, almond and apple and kiwi most commonly caused a severe reaction (51– 117 patients) due to the high number of patients reporting symptoms to these foods.

Table 2. Frequency and severity of foods reported to cause symptoms from most to least common

Rank	Patients with symptoms to	Frequency	Mild (%)	Moderate (%)	Severe (%)
1	Apple	482 (44.4)	55.5	33.0	11.1
2	Kiwi	374 (34.5)	54.8	30.7	14.5
3	Hazelnut	359 (33.1)	39.3	32.4	28.3
4	Walnut	325 (30)	38.0	36.6	25.4
5	Peanut	317 (29.2)	18.9	40.0	41.1
6	Cherry	258 (23.8)	49.2	35.3	15.5
7	Almond	221 (20.4)	35.0	35.9	29.1
8	Peach	213 (19.6)	48.3	37.2	14.5
9	Pear	207 (19.1)	52.3	34.5	13.2
10	Strawberry	171 (15.8)	44.3	37.3	18.4
11	Tomato	164 (15.1)	37.0	50.7	12.3
12	Cow's milk	145 (13.4)	9.5	75.9	14.6
13	Cashew nut	144 (13.3)	18.9	41.8	39.3
14	Carrot	130 (12)	45.6	35.2	19.2
15	Pineapple	127 (11.7)	55.5	31.1	13.4
16	Banana	123 (11.3)	46.1	37.4	16.5
16	Nectarine	123 (11.3)	47.9	38.7	13.4
17	Nuts unspecified	117 (10.8)	22.0	33.0	45.1
18	Pecan nut	94 (8.7)	26.0	42.9	31.2
19	Hen's egg	84 (7.7)	7.0	71.8	21.1
20	Plum	80 (7.4)	46.3	38.8	15.0
21	Shrimp	79 (7.3)	9.1	66.7	24.2
22	Sesame	77 (7.1)	25.8	36.4	37.9
23	Soy dairy	72 (6.6)	25.0	40.6	34.4
23	Pistachio	72 (6.6)	21.0	40.3	38.7
24	Macadamia nut	70 (6.5)	35.2	37.0	27.8
25	Brazil nut	66 (6.1)	31.5	37.0	31.5
26	Paprika	64 (5.9)	29.3	53.4	17.2
27	Mango	62 (5.7)	42.4	37.3	20.3
28	Pine nut	58 (5.3)	18.9	30.2	50.9
29	Avocado	57 (5.3)	40.0	49.1	10.9
30	Melon unspecified	52 (4.8)	54.2	20.8	25.0
31	Grape	49 (4.5)	30.4	45.7	23.9
32	Orange	46 (4.2)	24.4	61.0	14.6
32	Wheat	46 (4.2)	0.0	85.7	14.3
33	Apricot	44 (4.1)	50.0	34.1	15.9
34	Potato	36 (3.3)	24.2	54.5	21.2
35	Raspberry	33 (3)	50.0	33.3	16.7

Table 2. Frequency and severity of foods reported to cause symptoms from most to least common (*continued*)

Rank	Patients with symptoms to	Frequency	Mild (%)	Moderate (%)	Severe (%)
36	Celery	27 (2.5)	35.5	41.9	22.6
37	Soy unspecified	25 (2.3)	10.5	63.2	26.3
38	Galia melon	24 (2.2)	59.1	27.3	13.6
38	Mandarin	24 (2.2)	27.3	68.2	4.5
38	Fig	24 (2.2)	36.4	31.8	31.8
38	Sunflower seed	24 (2.2)	23.8	33.3	42.9
39	Pea	23 (2.1)	27.3	45.5	27.3
40	Mussel	22 (2)	10.5	73.7	15.8
41	Salmon	21 (1.9)	16.7	44.4	38.9
42	Eggplant	20 (1.8)	35.3	47.1	17.6
42	Lentils	20 (1.8)	16.7	61.1	22.2
43	Spinach	18 (1.7)	42.9	42.9	14.3
43	Pumpkin seed	18 (1.7)	40.0	46.7	13.3
43	Tuna	18 (1.7)	12.5	56.3	31.3
44	Mixed nuts	16 (1.5)	31.3	12.5	56.3
45	Green bean	15 (1.4)	28.6	42.9	28.6
45	White fish unspecified	15 (1.4)	21.4	42.9	35.7
45	Lobster	15 (1.4)	7.1	57.1	35.7
46	Chickpea	14 (1.3)	16.7	66.7	16.7
46	Soybean merged	14 (1.3)	27.3	54.5	18.2
46	Krab	14 (1.3)	15.4	61.5	23.1
46	Red meat	14 (1.3)	25.0	41.7	33.3
47	Watermelon	13 (1.2)	33.3	50.0	16.7
47	Pepper	13 (1.2)	0.0	77.8	22.2
48	Cucumber	12 (1.1)	36.4	18.2	45.5
48	Mustard	12 (1.1)	20.0	40.0	40.0
48	Codfish	12 (1.1)	0.0	66.7	33.3
48	Fish unspecified	12 (1.1)	12.5	37.5	50.0
49	Date	11 (1)	50.0	40.0	10.0
49	Jackfruit	11 (1)	27.3	27.3	45.5
49	Coconut	11 (1)	18.2	54.5	27.3
49	Poppy seed	11 (1)	20.0	60.0	20.0
50	Arugula	9 (0.8)	20.0	70.0	10.0
51	Pomgranate	9 (0.8)	44.4	22.2	33.3
51	Lettuce	9 (0.8)	0.0	28.6	71.4
51	Scallop	9 (0.8)	12.5	62.5	25.0
52	Berries unspecified	8 (0.7)	50.0	37.5	12.5

Table 2. Frequency and severity of foods reported to cause symptoms from most to least common (*continued*)

Rank	Patients with symptoms to	Frequency	Mild (%)	Moderate (%)	Severe (%)
52	**Cabbage**	8 (0.7)	0.0	75.0	25.0
52	**Herring**	8 (0.7)	14.3	28.6	57.1
52	**Squid**	8 (0.7)	37.5	37.5	25.0
53	**Citron**	7 (0.6)	28.6	71.4	0.0
53	**Blueberry**	7 (0.6)	57.1	42.9	0.0
53	**Bilberry**	7 (0.6)	0.0	83.3	16.7
53	**Blackberry**	7 (0.6)	66.7	16.7	16.7
53	**Radish**	7 (0.6)	42.9	28.6	28.6
53	**Oyster**	7 (0.6)	0.0	40.0	60.0
53	**Rice**	7 (0.6)	0.0	57.1	42.9
53	**Chicken**	7 (0.6)	28.6	57.1	14.3
54	**Garlic**	6 (0.6)	16.7	83.3	0.0
54	**Leek**	6 (0.6)	0.0	83.3	16.7
54	**Fennel**	6 (0.6)	16.7	33.3	50.0
54	**Soy sauce**	6 (0.6)	16.7	66.7	16.7
54	**Kidney beans**	6 (0.6)	0.0	83.3	16.7
54	**Rye**	6 (0.6)	0.0	66.7	33.3
56	**Red currant**	6 (0.6)	40.0	40.0	20.0
55	**Black currant**	5 (0.5)	0.0	50.0	50.0
55	**Zucchini**	5 (0.5)	40.0	40.0	20.0
55	**Iceberg lettuce**	5 (0.5)	25.0	50.0	25.0
55	**Beans unspecified**	5 (0.5)	0.0	80.0	20.0
55	**String bean**	5 (0.5)	40.0	40.0	20.0
55	**Broad bean**	5 (0.5)	50.0	0.0	50.0
55	**White bean**	5 (0.5)	0.0	60.0	40.0
55	**Mackerel**	5 (0.5)	25.0	0.0	75.0
55	**Eel**	5 (0.5)	0.0	100.0	0.0
55	**Oat**	5 (0.5)	0.0	75.0	25.0
56	**Passion fruit**	4 (0.4)	33.3	66.7	0.0
56	**Chestnut**	4 (0.4)	50.0	25.0	25.0
56	**Bean sprouts**	4 (0.4)	25.0	50.0	25.0
56	**Mushroom**	4 (0.4)	0.0	75.0	25.0
56	**Chili pepper**	4 (0.4)	0.0	100.0	0.0
56	**Chicory**	4 (0.4)	0.0	50.0	50.0
56	**Broccoli**	4 (0.4)	0.0	100.0	0.0
56	**Goat dairy**	4 (0.4)	0.0	50.0	50.0
56	**Ginger**	4 (0.4)	0.0	50.0	50.0

Table 2. Frequency and severity of foods reported to cause symptoms from most to least common (*continued*)

Rank	Patients with symptoms to	Frequency	Mild (%)	Moderate (%)	Severe (%)
56	**Cinnamon**	4 (0.4)	0.0	50.0	50.0
56	**Parsley**	4 (0.4)	25.0	25.0	50.0
57	**Honeydew melon**	3 (0.3)	33.3	66.7	0.0
57	**Grapefruit**	3 (0.3)	66.7	33.3	0.0
57	**Persimmon**	3 (0.3)	33.3	33.3	33.3
57	**Lychee**	3 (0.3)	0.0	33.3	66.7
57	**Goji berry**	3 (0.3)	33.3	66.7	0.0
57	**Asparagus**	3 (0.3)	0.0	33.3	66.7
57	**Pickle**	3 (0.3)	0.0	0.0	100.0
57	**Beet**	3 (0.3)	100.0	0.0	0.0
57	**Soy burger**	3 (0.3)	100.0	0.0	0.0
57	**Tempeh**	3 (0.3)	0.0	100.0	0.0
57	**Tofu**	3 (0.3)	0.0	100.0	0.0
57	**Capuchins**	3 (0.3)	50.0	50.0	0.0
57	**Trout**	3 (0.3)	33.3	33.3	33.3
57	**Buckwheat**	3 (0.3)	0.0	33.3	66.7
57	**Basil**	3 (0.3)	33.3	66.7	0.0
57	**Cilantro**	3 (0.3)	0.0	100.0	0.0
58	**Papaya**	2 (0.2)	0.0	50.0	50.0
58	**Cantaloupe**	2 (0.2)	100.0	0.0	0.0
58	**Mulberry**	2 (0.2)	50.0	0.0	50.0
58	**Elderberry**	2 (0.2)	0.0	50.0	50.0
58	**Beechnut**	2 (0.2)	0.0	50.0	50.0
58	**Corn**	2 (0.2)	0.0	50.0	50.0
58	**Parsnip**	2 (0.2)	50.0	50.0	0.0
58	**Rhubarb**	2 (0.2)	0.0	50.0	50.0
58	**Sweet potato**	2 (0.2)	0.0	50.0	50.0
58	**Cauliflower**	2 (0.2)	50.0	0.0	50.0
58	**Kale**	2 (0.2)	50.0	50.0	0.0
58	**Red cabbage**	2 (0.2)	50.0	50.0	0.0
58	**Soy flour**	2 (0.2)	0.0	100.0	0.0
58	**Soy powder**	2 (0.2)	0.0	100.0	0.0
58	**Pangasius**	2 (0.2)	50.0	0.0	50.0
58	**Sardine**	2 (0.2)	0.0	0.0	100.0
58	**Plaice**	2 (0.2)	50.0	50.0	0.0
58	**Sole**	2 (0.2)	0.0	50.0	50.0
58	**Langoustine**	2 (0.2)	0.0	100.0	0.0
58	**Clams**	2 (0.2)	0.0	100.0	0.0

Table 2. Frequency and severity of foods reported to cause symptoms from most to least common (*continued*)

Rank	Patients with symptoms to	Frequency	Mild (%)	Moderate (%)	Severe (%)
58	**Vongole**	2 (0.2)	0.0	100.0	0.0
58	**Spells**	2 (0.2)	0.0	100.0	0.0
58	**Turmeric**	2 (0.2)	50.0	0.0	50.0
58	**Paprika powder**	2 (0.2)	0.0	100.0	0.0
58	**Truffle**	2 (0.2)	0.0	50.0	50.0
58	**Rosemary**	2 (0.2)	0.0	0.0	100.0
58	**Thyme**	2 (0.2)	0.0	0.0	100.0
59	**Lemon**	1 (0.1)	0.0	100.0	0.0
59	**Inca berries**	1 (0.1)	100.0	0.0	0.0
59	**Caper berry**	1 (0.1)	0.0	100.0	0.0
59	**Gooseberry**	1 (0.1)	0.0	100.0	0.0
59	**Myrica Rubra**	1 (0.1)	0.0	100.0	0.0
59	**Kemiri nut**	1 (0.1)	0.0	100.0	0.0
59	**Caper**	1 (0.1)	0.0	100.0	0.0
59	**Endive**	1 (0.1)	0.0	0.0	100.0
59	**Oak leaf lettuce**	1 (0.1)	100.0	0.0	0.0
59	**Lamb's lettuce**	1 (0.1)	100.0	0.0	0.0
59	**Turnip cabbage**	1 (0.1)	100.0	0.0	0.0
59	**Turnip greens**	1 (0.1)	100.0	0.0	0.0
59	**Sprouts**	1 (0.1)	0.0	100.0	0.0
59	**Sauerkraut**	1 (0.1)	0.0	100.0	0.0
59	**Lupine**	1 (0.1)	0.0	100.0	0.0
59	**Asparagus bean**	1 (0.1)	0.0	100.0	0.0
59	**Sugar snap**	1 (0.1)	0.0	0.0	100.0
59	**Tamarind**	1 (0.1)	0.0	100.0	0.0
59	**Black beans**	1 (0.1)	0.0	100.0	0.0
59	**Sheep dairy**	1 (0.1)	0.0	0.0	100.0
59	**Flax seed**	1 (0.1)	0.0	0.0	100.0
59	**Hake**	1 (0.1)	0.0	100.0	0.0
59	**Red snapper**	1 (0.1)	0.0	100.0	0.0
59	**Redfish**	1 (0.1)	0.0	100.0	0.0
59	**Small sole fish**	1 (0.1)	0.0	0.0	100.0
59	**Tilapia**	1 (0.1)	100.0	0.0	0.0
59	**Sea bass**	1 (0.1)	0.0	100.0	0.0
59	**Swordfish**	1 (0.1)	0.0	0.0	100.0
59	**Anise**	1 (0.1)	100.0	0.0	0.0
59	**Clove**	1 (0.1)	0.0	100.0	0.0

Table 2. Frequency and severity of foods reported to cause symptoms from most to least common (*continued*)

Rank	Patients with symptoms to	Frequency	Mild (%)	Moderate (%)	Severe (%)
59	**Nutmeg**	1 (0.1)	0.0	100.0	0.0
59	**Saffron**	1 (0.1)	0.0	100.0	0.0
59	**Liquorice**	1 (0.1)	100.0	0.0	0.0
59	**Mint**	1 (0.1)	0.0	100.0	0.0
59	**Cricket**	1 (0.1)	0.0	0.0	100.0

Color legend:
- Food: Crustaceans; meat; mollusks; fish; nuts; seed or pits; grains; insects; fruit; vegetables; legumes; spices and herbs; dairy; hen's egg
- Number of patients that reported mild, moderate or severe symptoms: 0.0%; 100.0%

For the severity score, only patients with a known severity score were included.

Food groups

The 192 foods were classified into 14 food groups (**Figure 1**). Plant-based foods most commonly caused symptoms: 69.8% of the patients reported symptoms to fruit, 63.0% to tree nuts, 39.5% to legumes, 34.0% to vegetables and 14.4% to seeds and pits. These were followed by the animal foods dairy (13.7%), hen's egg (7.7%), crustaceans (7.6%) and fish (6.8%). Fewer patients reported symptoms to grains (5.8%), mollusks (3.9%), spices and herbs (3.4%), meat (1.7), and insects (0.1%).

Regarding severity, seeds and pits (39.8%) and fish (39.2%) most commonly caused severe reactions, followed by legumes, nuts and crustaceans, to which about a third of patients reported severe reactions. (**Figure 1**). When looking at absolute patient numbers, severe reactions were most frequently seen for nuts (N=252), legumes (N=218) and fruits (N=149). So, in absolute patient numbers, fruit belongs to one of the food groups most frequently causing severe reactions even though it is generally seen as a food causing mild symptoms.

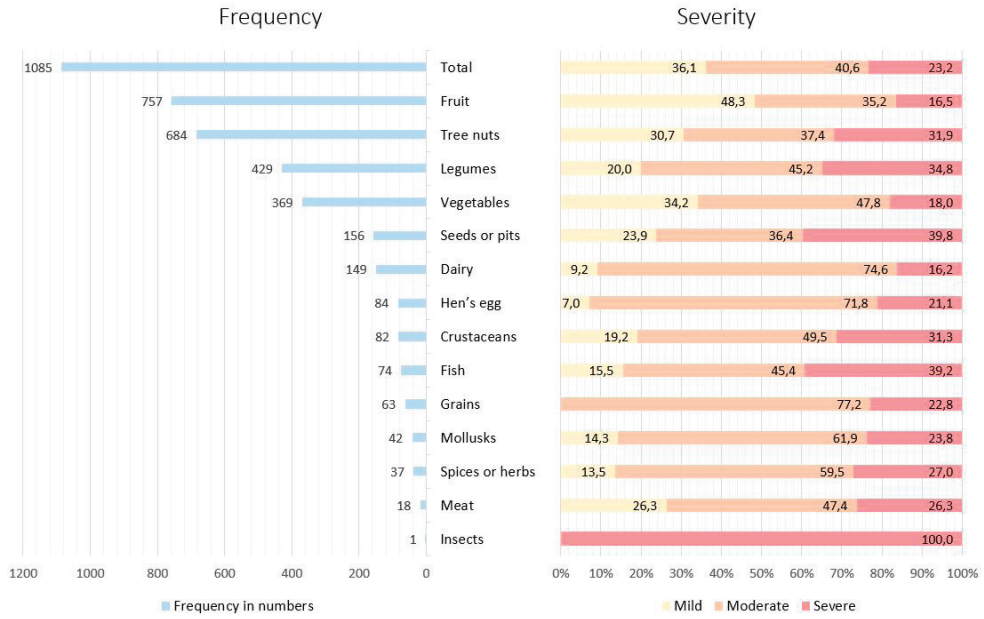


Figure 1. Frequency and severity of food groups reported to cause symptoms from least to most common

Patients could report symptoms for foods in more than one group. Therefore, the total number of patients does not equal the sum of patients with symptoms for each food groups.

For the severity score, only patients with a known severity score were included.

Tree nuts and legumes further specified

Food groups most frequently causing severe symptoms in absolute numbers were tree nuts and legumes. Therefore, these two groups are described in more detail.

The within-group differences in frequency were large for both groups, but the within-group differences in severity were small for tree nuts and large for legumes. Frequency and severity for each tree nut are shown in **Table 3**. Symptoms to hazelnut were most common (52.4%), followed by walnut (47.5%), almond (32.2%), cashew nut (21.1%) and pecan nut (13.7%). Symptoms to Brazil nut, coconut, chestnut, beech nut and kemiri nut were less common (<10%). Based on percentages, there was no large difference in severity, with almost all tree nuts causing severe symptoms in one third of the patients.

Frequency and severity of legumes are shown in **Table 3**. Symptoms to peanuts were by far most common (73.9%), followed by soy dairy (16.8%) and other soy products (12.1%). Symptoms to bean, pea, lentil, chickpea and lupine were less common (<10%). Peanut most often caused a severe reaction (41.1%), followed by soy dairy (34.4%), bean (27.9%), pea (25.0%), and lentil (22.2%). However, several foods did not cause any severe symptoms, particularly the more processed soy products like soy burger and tempeh, but

also asparagus bean, tamarind, black bean, capuchins, and lupine. More than 1,5 times as many patients had severe symptoms to soy dairy than to other soy products.

In conclusion, both tree nuts and legumes most commonly caused severe symptoms, with almost all tree nuts causing severe symptoms in 30% of patients and legumes having a wider distribution of severity.

Other food groups further specified

Detailed information about the frequency and severity of the other food groups can be found in the Supplementary file (**Table S1.1-S1.12**). The most notable results will be discussed below.

The fruits to which patients most frequently reported severe reactions were mostly tropical, with a severe reaction to lychee in 66.7%, papaya in 50.0%, jackfruit in 45.5%, both persimmon and pomegranate in 33.3%, fig in 31.8% and mango in 20.3%. Except for mango and fig, these were fruits for which patients were less likely to report symptoms to (N < 15).

When patients reported symptoms to seeds and pits, it was mostly to sesame (N=77) and pine nuts (N=58) with severe symptoms in 37.9% and 50.9% respectively. Symptoms to sunflower seeds and mustard were less common (N = 24 and N = 12, respectively), but quite as severe with 42.9% and 40.0% of the patients reporting symptoms, respectively.

Table 3. Frequency and severity of reported symptoms most to least common in 684 patients to tree nuts and 429 patients to legumes

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Tree nuts	***N = 684***			
Hazelnut	359 (52.4)	39.3	32.4	28.3
Walnut	325 (47.5)	38.0	36.6	25.4
Almond	221 (32.3)	35.0	35.9	29.1
Cashew nut	144 (21.1)	18.9	41.8	39.3
Unspecified nuts	117 (17.1)	22.0	33.0	45.1
Pecan nut	94 (13.7)	26.0	42.9	31.2
Pistachio	72 (10.5)	21.0	40.3	38.7
Macadamia nut	70 (10.2)	35.2	37.0	27.8
Brazil nut	66 (9.6)	31.5	37.0	31.5
Mixed nuts	16 (2.3)	31.3	12.5	56.3
Coconut	11 (1.6)	18.2	54.5	27.3
Chestnut	4 (0.6)	50.0	25.0	25.0
Beechnut	2 (0.3)	0.0	50.0	50.0
Kemiri nut	1 (0.1)	0.0	100.0	0.0

Table 3. Frequency and severity of reported symptoms most to least common in 684 patients to tree nuts and 429 patients to legumes (*continued*)

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Legumes	***N = 429***			
Peanut merged	317 (73.9)	18.9	40.0	41.1
Soy dairy	72 (16.8)	25.0	40.6	34.4
Soy other	52 (12.1)	18.2	63.6	18.2
Soy unspecified	25 (5.8)	10.5	63.2	26.3
Soybean	14 (3.3)	27.3	54.5	18.2
Soy sauce	6 (1.4)	16.7	66.7	16.7
Soy burger	3 (0.7)	100.0	0.0	0.0
Tempeh	3 (0.7)	0.0	100.0	0.0
Tofu	3 (0.7)	0.0	100.0	0.0
Soy flour	2 (0.5)	0.0	100.0	0.0
Soy powder	2 (0.5)	0.0	100.0	0.0
Bean	36 (8.4)	18.6	53.5	27.9
Green bean	15 (3.5)	28.6	42.9	28.6
Kidney beans	6 (1.4)	0.0	83.3	16.7
Beans unspecified	5 (1.2)	0.0	80.0	20.0
String bean	5 (1.2)	40.0	40.0	20.0
Broad bean	5 (1.2)	50.0	0.0	50.0
White bean	5 (1.2)	0.0	60.0	40.0
Asparagus bean	1 (0.2)	0.0	100.0	0.0
Sugar snaps	1 (0.2)	0.0	0.0	100.0
Tamarind	1 (0.2)	0.0	100.0	0.0
Black beans	1 (0.2)	0.0	100.0	0.0
Pea	23 (5.4)	29.2	45.8	25.0
Green pea	23 (5.4)	27.3	45.5	27.3
Capuchins	3 (0.7)	50.0	50.0	0.0
Lentils	20 (4.7)	16.7	61.1	22.2
Chickpea	14 (3.3)	16.7	66.7	16.7
Lupine	1 (0.2)	0.0	100.0	0.0

Patients could report symptoms for more than one tree nut. Therefore, the total number of patients does not equal the sum of patients with symptoms for each tree nut.
For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Co-allergies

Co-allergies between food groups and within food groups are shown in **Table 4-5 and S2.1-4 and Figure 2**. The most remarkable results will be described in more detail.

The frequency of co-allergies between the different seeds and pits were in general low (<45%) (**Figure 2, Table S2.1**). Adult patients with symptoms to sesame had low frequency of co-allergies to other seeds and pits, with co-allergy for pine nut being most common (20.8%). The highest rate of co-allergies were seen for sunflower seed and poppy seed with sesame (41.7% and 45.5%, respectively), sunflower seed with pine nut (37.5%) and pumpkin seed with sunflower seed (38.9%). Of the patients with symptoms to seeds and pits, 79.5% also reported symptoms to tree nuts. Conversely, only 18.1% of patients with symptoms to tree nuts also reported symptoms to seeds and pits.

Table 4. Frequency of patients with symptoms to a food group and the percentage of patients with co-allergies to other food groups

When symptoms to …		% of patients with also symptoms to													
	N	Fruit	Nuts	Legumes	Vegetables	Seeds or pits	Milk	Hens' egg	Crustaceans	Fish	Grains	Mollusks	Spices or herbs	Meat	Insect
Fruit	757		65.7	41.0	41.9	14.0	12.4	7.5	6.3	6.3	5.4	3.6	4.0	1.8	0.0
Nuts	684	72.7		45.3	37.1	18.1	13.0	7.9	6.3	6.7	5.1	3.4	3.1	1.8	0.0
Legumes	429	72.3	72.3		41.0	19.1	16.8	12.8	6.3	6.5	8.6	3.0	3.5	2.1	0.0
Vegetables	369	85.9	68.8	47.7		21.4	13.6	8.9	8.9	6.2	7.6	4.1	5.4	2.4	0.0
Seeds and pits	156	67.9	79.5	52.6	50.6		11.5	11.5	7.7	10.3	9.0	4.5	5.1	2.6	0.0
Milk	149	63.1	59.7	48.3	33.6	12.1		16.8	6.0	8.7	12.1	4.0	4.0	2.7	0.0
Hens' egg	84	67.9	64.3	65.5	39.3	21.4	29.8		6.0	11.9	13.1	8.3	3.6	8.3	0.0
Crustaceans	82	58.5	52.4	32.9	40.2	14.6	11.0	6.1		18.3	9.8	18.3	7.3	3.7	0.0
Fish	74	64.9	62.2	37.8	31.1	21.6	17.6	13.5	20.3		12.2	14.9	6.8	2.7	0.0
Grains	63	65.1	55.6	58.7	44.4	22.2	28.6	17.5	12.7	14.3		9.5	6.3	3.2	0.0
Mollusks	41	65.9	56.1	31.7	36.6	17.1	14.6	17.1	36.6	26.8	14.6		4.9	4.9	2.4
Spices and herbs	37	81.1	56.8	40.5	54.1	21.6	16.2	8.1	16.2	13.5	10.8	5.4		0.0	0.0
Meat	18	77.8	66.7	50.0	50.0	22.2	22.2	38.9	16.7	11.1	11.1	11.1	0.0		0.0
Insect	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	

Color legend: ○ 0.0% co-allergies; ● 100.0% co-allergies

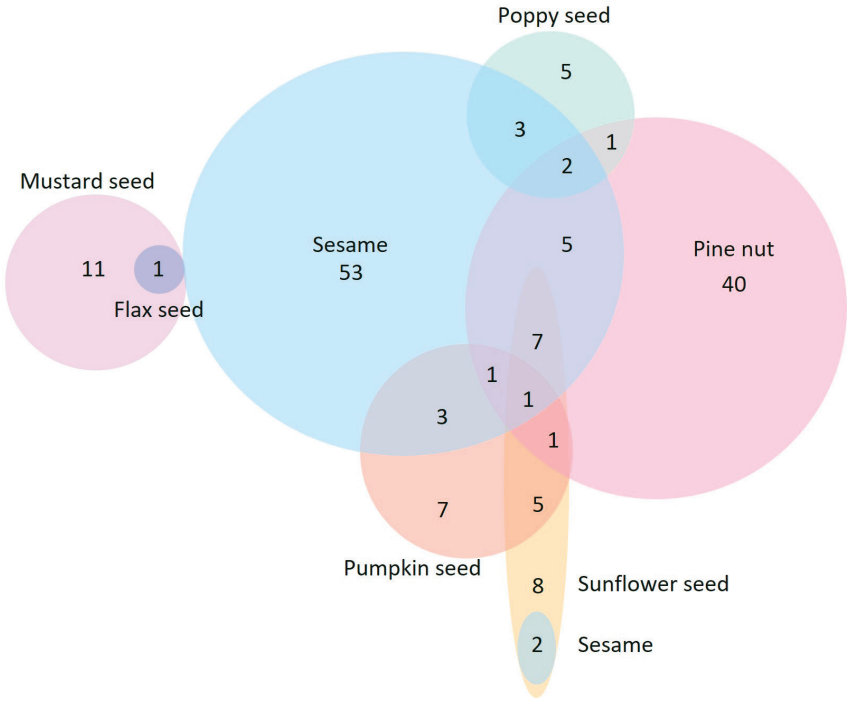
In the legume group, patients who reported symptoms to soy, beans, peas, lentils, and chickpea often had a co-allergy for peanut (23.6% - 47.8%) (**Table V**). Contrarily, patients who reported symptoms to peanut less often had a co-allergy for the other legumes (0% - 5.7%). Co-allergies between beans, peas, lentils, and chickpeas ranged between 11.1% - 55.0%.

Table 5. Frequency of reported symptoms to legumes with co-allergies to other legumes

When symptoms to …		% of patients with also symptoms to							
	N	**Peanuts**	**Soy dairy**	**Soy other**	**Beans**	**Peas**	**Lentils**	**Chickpea**	**Lupine**
Peanuts	317		5.4	5.7	3.8	3.5	2.8	1.3	0.0
Soy dairy	72	23.6		15.3	6.9	2.8	1.4	1.4	0.0
Soy other	52	34.6	21.2		11.5	5.8	13.5	7.7	1.9
Beans	36	33.3	13.9	16.7		27.8	25.0	11.1	2.8
Peas	23	47.8	8.7	13.0	43.5		47.8	17.4	4.3
Lentils	20	45.0	5.0	35.0	45.0	55.0		35.0	5.0
Chickpea	14	28.6	7.1	28.6	28.6	28.6	50.0		7.1
Lupine	1	0.0	0.0	100.0	100.0	100.0	100.0	100.0	

*Soy other: soy unspecified, soybean, soy sauce, soy burger, tempeh, tofu, soy flour and soy powder
Color legend: ○ 0.0% co-allergies; ● 100.0% co-allergies

Figure 2. Co-allergy in patients with reported symptoms to seeds or pits.



Of the patients reporting symptoms to tree nuts, 29.5%-52.8% of patients also did to peanuts, but among patients with symptoms to peanuts, only 7.9%-34% of the patients also did to tree nuts, mainly to hazelnut, walnut and almond (**Table S2.2**).

Within the fruit and vegetable group, there were relatively fewer co-allergies than in the other food groups (**Table S2.3 and S2.4**). Related foods showed often co-allergies. This was particularly noticeable for fruits like *Rosaceae*, berries and citrus fruit. Other noteworthy co-allergies were seen between the tropical fruit mango and jackfruit, persimmon and lychee, and fig and date.

Patients with a probable food allergy

Enough sensitization data was available for 45 foods. However, the ranking in frequency and severity of the different foods for patients with probable and possible FA were quite comparable (**Table 6**). The most notable results will be discussed below.

Frequency and severity of allergic reactions

Of the patients with a probable FA, apple allergy was most common (37.2%), followed by hazelnut (30.5%), peanut (24.8%), kiwi (22.0%), peach (15.9%), walnut (13.4%), and almond (12.7%). Almost 70% of the food allergic reactions were caused by these seven foods and 30% by the remaining 38 foods. Although mandatory labeled, a probable FA was uncommon for salmon (0.7%), codfish (0.6%), lobster (0.4%), krab (0.3%), and tuna (0.2%).

When focusing on the frequency of severe symptoms of the individual foods for which at least 5 patients were allergic, sunflower seeds, pine nut, Brazil nut, cashew nut, and pistachio most frequently caused severe symptoms (51.7-80.0%). However, in absolute numbers, peanut, hazelnut, apple, walnut, and almond most commonly caused a severe reaction (33-84 patients) due to the high numbers of patients reporting symptoms to these foods.

For 64.0% of the foods that were reported less than 15 times, patients reported more frequently moderate or severe symptoms than mild symptoms, while this percentage was 36.4% for foods that were reported at least 50 times. This indicates that patients are more likely to have a more severe reaction to foods that less frequently cause symptoms.

Table 6. Frequency with corresponding severity of patients with a probable food allergy (symptoms and sensitization) or with a possible food allergy (only reported symptoms)

	Probable food allergy				Possible food allergy			
Food	Frequency	Mild (%)	Moderate (%)	Severe (%)	Frequency	Mild (%)	Moderate (%)	Severe (%)
Apple	352 (37.2)	56.2	31.8	12.1	482 (44.4)	55.5	33.0	11.1
Hazelnut	288 (30.5)	38.3	30.9	30.9	359 (33.1)	39.3	32.4	28.3
Peanut	234 (24.8)	17.7	42.1	40.2	317 (29.2)	18.9	40.0	41.1
Kiwi	208 (22)	52.3	32.2	15.6	374 (34.5)	54.8	30.7	14.5
Peach	150 (15.9)	47.9	35.6	16.4	213 (19.6)	48.3	37.2	14.5
Walnut	127 (13.4)	29.6	37.4	33.0	325 (30)	38.0	36.6	25.4
Almond	120 (12.7)	33.9	36.6	29.5	221 (20.4)	35.0	35.9	29.1
Carrot	66 (7)	50.0	30.3	19.7	130 (12)	45.6	35.2	19.2
Tomato	59 (6.2)	44.4	42.6	13.0	164 (15.1)	37.0	50.7	12.3
Soy dairy	54 (5.7)	22.9	33.3	43.8	72 (6.6)	25.0	40.6	34.4
Cashew nut	50 (5.3)	4.8	38.1	57.1	144 (13.3)	18.9	41.8	39.3
Sesame	46 (4.9)	24.4	31.7	43.9	77 (7.1)	25.8	36.4	37.9
Hen's egg	39 (4.1)	6.9	75.9	17.2	84 (7.7)	7.0	71.8	21.1
Shrimp	35 (3.7)	29.0	32.3	38.7	79 (7.3)	22.9	44.3	32.9
Pistachio	35 (3.7)	13.8	34.5	51.7	72 (6.6)	21.0	40.3	38.7
Cherry	34 (3.6)	45.5	27.3	27.3	258 (23.8)	49.2	35.3	15.5
Banana	30 (3.2)	34.5	51.7	13.8	123 (11.3)	46.1	37.4	16.5
Cow's milk	29 (3.1)	8.0	60.0	32.0	145 (13.4)	9.5	75.9	14.6
Paprika	19 (2)	38.9	38.9	22.2	64 (5.9)	29.3	53.4	17.2
Macadamia nut	16 (1.7)	58.3	8.3	33.3	70 (6.5)	35.2	37.0	27.8
Pine nut	16 (1.7)	6.7	26.7	66.7	58 (5.3)	18.9	30.2	50.9
Pecan nut	15 (1.6)	9.1	45.5	45.5	94 (8.7)	26.0	42.9	31.2
Brazil nut	14 (1.5)	10.0	30.0	60.0	66 (6.1)	31.5	37.0	31.5
Pear	13 (1.4)	83.3	0.0	16.7	207 (19.1)	52.3	34.5	13.2
Soy other	13 (1.4)	30.0	50.0	20.0	52 (4.8)	18.2	63.6	18.2
Pea	12 (1.3)	25.0	33.3	41.7	23 (2.1)	27.3	45.5	27.3
Wheat	11 (1.2)	0.0	57.1	42.9	46 (4.2)	0.0	85.7	14.3
Celery	9 (1.0)	57.1	28.6	14.3	27 (2.5)	36.0	48.0	16.0
Avocado	8 (0.8)	75.0	12.5	12.5	57 (5.3)	40.0	49.1	10.9
Salmon	7 (0.7)	16.7	50.0	33.3	21 (1.9)	16.7	44.4	38.9
Potato	6 (0.6)	0.0	100.0	0.0	36 (3.3)	24.2	54.5	21.2
Lentils	6 (0.6)	16.7	50.0	33.3	20 (1.8)	16.7	61.1	22.2
Codfish	6 (0.6)	0.0	60.0	40.0	12 (1.1)	0.0	66.7	33.3
Strawberry	5 (0.5)	25.0	25.0	50.0	171 (15.8)	44.3	37.3	18.4
Sunflower seed	5 (0.5)	20.0	0.0	80.0	24 (2.2)	23.8	33.3	42.9

Table 6. Frequency with corresponding severity of patients with a probable food allergy (symptoms and sensitization) or with a possible food allergy (only reported symptoms) (*continued*)

	Probable food allergy				Possible food allergy			
Spinach	4 (0.4)	50.0	50.0	0.0	18 (1.7)	42.9	42.9	14.3
Lobster	4 (0.4)	0.0	50.0	50.0	15 (1.4)	7.1	57.1	35.7
Orange	3 (0.3)	33.3	66.7	0.0	46 (4.2)	24.4	61.0	14.6
Krab	3 (0.3)	33.3	33.3	33.3	14 (1.3)	15.4	61.5	23.1
Melon	2 (0.2)	0.0	0.0	100.0	52 (4.8)	55.3	21.3	23.4
Mussel	2 (0.2)	100.0	0.0	0.0	22 (2)	10.5	73.7	15.8
Tuna	2 (0.2)	0.0	50.0	50.0	18 (1.7)	12.5	56.3	31.3
Pineapple	1 (0.1)	100.0	0.0	0.0	127 (11.7)	55.5	31.1	13.4
Chickpea	1 (0.1)	0.0	100.0	0.0	14 (1.3)	16.7	66.7	16.7
Chicken	1 (0.1)	100.0	0.0	0.0	7 (0.6)	28.6	57.1	14.3

For the severity score. only patients with a known severity score were included
Color legend: Number of patients that reported mild. moderate or severe symptoms: ○ 0.0%; ● 100.0%

Association between sensitization pattern and severity in plant-based food allergy

Sensitization patterns were measured by immunoblot from March 2022. From this date, a total of 358 patients reported symptoms to foods, of whom 337 reported symptoms to plant based food, and in 291 patients sensitization was measured by immunblot. Of the 291 patients in whom sensitization was measured, 78.7% patients were sensitized to PR-10, 26.8% to SS, 10.7% to LTP and 15.5% were not sensitized to PR-10, SS and LTP. Mono-sensitization to PR-10 was the most common, occurring in half of the patients. For SS and LTP, mono-sensitization occurred in 4.1% and 1.0% of patients, respectively. In total, 27.8% of the patients recognized a combination of the three allergen groups of which sensitization to PR-10/SS was most common with 65.4%.

The severity of reactions for the different sensitization groups is shown in **Table S3**. There was no significant difference in severity between the groups (p=0.278). Severe reactions were seen in 32.7% of the patients in the PR-10 group and in 50.0% of the patients in the SS group. In the PR-10/SS combination group, 35.8% had a severe reaction, which was higher than the pure PR-10 group, but lower than the pure SS group. The LTP group was too small for comparison.

For only four foods, CRD was measured for multiple allergen families, namely hazelnut, peanut, apple, and kiwi. For hazelnut and apple, around 80% of the patients were mono-sensitized to PR-10, and for peanut and kiwi, this was 44.9% and 58.1% respectively (**Table S4**). For hazelnut and peanut, patients mono-sensitized to non-PR-10 components had significantly more commonly severe symptoms compared to patients mono-sensitized to PR-10 components. However, this was not seen for apple and kiwi. For apple and kiwi,

there was a numerical trend towards more severe symptoms for those mono-sensitized to the PR-10 components than those mono-senzitized to non-PR-10 components.

To conclude, around 80% of our study population recognized PR-10 proteints which is explained by the location in a birch endemic area. Sensization to non-PR-10 proteins cause, especially in peanut and hazelnut, more often severe symptoms compared to sensitization to PR-10 proteins. However, also PR-10 monosensitization can cause severe symptoms.

DISCUSSION

This is the most comprehensive study to date investigating almost 200 foods that caused a possible FA and 45 foods that caused a probable FA in an outpatient allergology clinic. Although data on probable FA is preferred over possible FA, the ranking of foods in terms of frequency and severity was quite comparable in both groups. Therefore, to get the best estimate of all foods and food groups that contribute to the burden of food allergy, we included also foods causing possible FA. Almost 80% of the reported symptoms were caused by only 30 foods. Fruit, tree nuts, and legumes most commonly caused an allergy and insects, meat, and spices or herbs least commonly. Severe symptoms were most frequently reported for seed or pits, nuts, and legumes. Fruit was one of the most common causes of a severe reaction due to the high frequency of symptoms to these foods. The differences in severity were large for the different legumes, whereas in tree nuts there was no large difference in severity. Peanut allergic patients often did not reported symptoms to other legumes, whereas patients with symptoms to other legumes often reacted to peanut.

Fruit and vegetables are relevant food allergies

The food groups that most commonly caused symptoms were all plant-based foods, with fruits, tree nuts and legumes ranking highest. It is difficult to compare our data with other studies, because, as far as we know, there are no studies that have included so many foods. The EuroPrevall study investigated the frequency of 24 priority foods and other foods that caused symptoms between 2006-2009.[12] We performed a post hoc analysis with the Dutch EuroPrevall outpatient clinic data for comparison (**Table S5**). Results were largely comparable: the 17 most commonly reported foods were similar (except for cow's milk). Fruit, nuts, peanut, and vegetables topped the list. This confirms that foods outside the big eight, especially fruits and vegetables, are also important food allergies.

The most noticeable difference was that more than twice as many patients in our study reported symptoms to tree nuts, pits, and tropical fruit compared to the EuroPrevall study. This might be explained by the change in consumption. Dutch consumption data

showed a shift towards a more plant-based diet between 2007-2010 and 2019-2021: an increase of > 20% of the consumption of legumes, fruit, vegetables, tree nuts and seeds.[13] In our study, around 85% had a probable plant food allergy, which was mainly associated with birch pollen-associated food allergy. The change in diet over years, and therefore the potential change in allergy, warrants regular monitoring of food allergy over the years.[14]

Foods that are mandatory labeled not always cause frequent or severe symptoms and vice versa

Mandatory labelling is meant to protect allergic consumers.[15] There are still undeclared allergens that potentially cause serious health risks.[16,17] The frequency from high to low of our adult patients to the 14 labelled food(group)s[18] was as follows (respectively possible FA/probable FA): 1) tree nuts 52.6%/41.5%; 2) peanut 29.2%/24.9%; 3) cow's milk 13.4%/3.1%; 4) soy 10.4%/6.7%; 5) hen's egg 7.7%/4.1%; 6) crustaceans 7.6%/4.2%; 7) sesame 7.1%/4.9%; 8) fish 6.8%/2.8%; 9) cereal 5.3%/1.2%; 10) celery 2.5%/1.0; 11) mustard 1.1%/NA; 12) mollusks 0.7%/NA; 13) lupin 0.1%/NA; and 14) sulfur dioxide (additive) uncommon. When comparing the 14 foods that require labeling with our results, it is noticeable that some foods causing frequent or severe reactions are not mentioned and vice versa. For example, we have shown that pine nut and sunflower seed (both for patients with a probable and possible FA) most frequently cause severe symptoms and are not labelled, whereas symptoms were neither frequent nor (often) severe for lupine, fish, crustaceans other than shrimp, mustard, and celery. As seeds and pits are not only consumed as a whole, but are widely used in industrially produced compound foods, further attention needs to be paid to their relevance as a potential hidden allergen.

It is important to note that these numbers represents the patients in one European country, whereas food labeling applies across the whole European Union where the severity could be different elsewhere. For example, a celery allergy in Swiss is severe in 31.6% of patients.[19] This highlights the importance of prevalence and severity data for all food allergies for appropriate labelling in different countries.

Common fruits cause severe reactions in high absolute numbers and tropical fruits in high percentage

Fruit was one of the most common causes of a severe reaction due to the high frequency of symptoms to these foods. Although literature state that fruit allergy is mostly mild, there are several studies that showed that it can also be severe.[20–22] A study of 218 cases of food-induced anaphylaxis showed that fruits can cause anaphylaxis with pitted fruit (not further specified), kiwi and banana being the culprit foods.[23] Another study investigating 250 cases of fruit-only induced anaphylaxis, showed that kiwi, banana and mango were the leading cause of anaphylaxis.[22] These reports are roughly in line with our findings, with kiwi, apple, cherry, peach, strawberry and banana being the most frequently reported fruits in absolute terms that are linked to severe symptoms.

In relative terms, our adult patients reported most frequently severe symptoms to tropical fruits such as lychee, papaya, jackfruit, persimmon, pomegranate, fig, and mango. It is expected that consumption of exotic fruits will rise due to an increased availability by import[24], a growing interest in new flavors and fruit varieties[24] and traveling. This may cause a rise in frequency and severity of food allergies to exotic fruits.

Large differences in frequency and severity of food allergy within the legume group

Since peanut allergy is one of the most frequent and severe food allergies, it is important to know whether consumption of other legumes, poses peanut allergic patients at high risk of a reaction. This is especially important, because legumes are an important alternative protein source and it is expected that consumption will rise in the future. We showed that only a minority of patients who reported symptoms to peanut also reacted to other legumes. However, patients who reacted to other legumes, often also had symptoms to peanut. Within the legume group, there were large differences in frequency and severity of an allergy to the individual legumes.

Seeds or pits: most severe food group, co-allergies between seeds and pits variable

Seeds or pits was the food group that most commonly caused severe symptoms. We have shown that patients with symptoms to seeds or pits often also reported symptoms to tree nuts (in 80%), but not vice versa (18%). Studies of co-allergies among tree nuts and seeds are limited. Brough et al. showed that patients with a positive OFC to pine nut and sesame were more likely to have a positive OFC to tree nuts (8.3-83.3%) than vice versa (9.6-28.6%).[25] This is in agreement with our findings. So, patients reporting symptoms to seeds and pits have a higher chance of symptoms to tree nuts, than the other way around.

Less is known about co-allergies between seeds and pits. We showed that when patients reported symptoms to seeds or pits, they most often also reported symptoms to sesame. Other common co-allergies were between pumpkin seed and sunflower seed (38.9%) and sunflower seed with pine nut (37.5%). More research is needed to determine the clinical reactivity between seeds and pits, especially since these have become increasingly popular due to their favorable nutritional profile.[26]

Strengths and limitations

The main limitation of this study was that we only had enough sensitization data for 45 foods, rather than for all 192 foods, and that OFC data was not included. Therefore, the results should be interpreted with caution. Nevertheless, the availability of sensitization data for 45 foods is quite substantial. Moreover, the majority of patients with possible FA also had probable FA, so they are experienced in recognizing and reporting food allergic symptoms. Unfortunately, for many foods no commercial diagnostic test are available

and PTPs are not standardised. In addition, especially for lesser common foods the diagnostic value of sIgE tests is unknown, and false negative results are not uncommon.[8,27,28] Because we are particularly interested in the prevalence of these less common foods and novel foods, we also investigated possible food allergy. A previous pan-European study investigated the predictive value of patient history and showed that reporting of oral allergy symptoms and allergic rhinitis comorbidity were strong predictors of probable FA. In our outpatient clinic, highly trained food allergy specialist took the patient history and because in our study population, 85.7% reported allergic rhinitis, 75% reported oral allergy symptoms and in addition we only selected patients who reacted within 2 hours, with even 83.5% who reacted within 5 minutes after ingesting the food, food allergy is likely in the majority of our patients.[9] Furthermore, we also investigated patients with a probable food allergy and showed that the frequency of severity of the symptoms of the patients with a probable food allergy was comparable to that of the patients with possible FA. Another limitation was that we excluded patients who reported symptoms after ingestion of a composite meal where the causative food ingredient was unclear, which could give rise to information bias. However, the potential bias was limited because most of the causative food ingredients were known for most patients. Lastly, the retrospective design of this daily practice study resulted in missing data, e.g. regarding sensitization, but the inclusion of over a thousand patients ensured a rich and representative dataset.

As far as we know, this is the first study that provides a comprehensive overview of all the foods that cause symptoms with corresponding severity in more than a thousand patients. We focused not only on the Big Eight foods, but on all foods that cause symptoms, including the less commonly reported and novel foods.

Conclusions

Food allergy can be caused by almost 200 different foods in adult patients. Seeds or pits, which are not mandatory labeled, most frequently cause severe symptoms and are a common food allergy. Large differences in frequency and severity of FA were observed within the legume group. In absolute numbers, fruit is one of the most common food causing severe reactions. Especially tropical fruit causing severe symptoms in high percentage of patients. This study highlights the need for more knowledge about food allergies outside the Big Eight, preferably in a prospective study design.

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SUPPLEMENTAL

Table S1.1 Frequency and severity of reported symptoms to fruit from most to least common in 757 patients

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Apple	482 (63.7)	55.5	33.0	11.1
Kiwi	374 (49.4)	54.8	30.7	14.5
Cherry	258 (34.1)	49.2	35.3	15.5
Peach	213 (28.1)	48.3	37.2	14.5
Pear	207 (27.3)	52.3	34.5	13.2
Strawberry	171 (22.6)	44.3	37.3	18.4
Pineapple	127 (16.8)	55.5	31.1	13.4
Banana	123 (16.2)	46.1	37.4	16.5
Nectarine	123 (16.2)	47.9	38.7	13.4
Melon total	95 (12.5)	38.4	42.8	18.8
Melon unspecified	*52 (6.9)*	54.2	20.8	25.0
Galia melon	*24 (3.2)*	59.1	27.3	13.6
Watermelon	*3 (0.4)*	33.3	50.0	16.7
Honeydew melon	*13 (1.7)*	33.3	66.7	0.0
Cantaloupe	*2 (0.3)*	100.0	0.0	0.0
Plum	80 (10.6)	46.3	38.8	15.0
Mango	62 (8.2)	42.4	37.3	20.3
Berries total	51 (6.7)	36.8	44.2	19.0
Berries unspecified	*8 (1.1)*	50.0	37.5	12.5
Blueberry	*7 (0.9)*	57.1	42.9	0.0
Bilberry	*7 (0.9)*	0.0	83.3	16.7
Blackberry	*7 (0.9)*	66.7	16.7	16.7
Current	*6 (0.8)*	40.0	40.0	20.0
Black currant	*5 (0.7)*	0.0	50.0	50.0
Goji berry	*3 (0.4)*	33.3	66.7	0.0
Mulberry	*2 (0.3)*	50.0	0.0	50.0
Elderberry	*2 (0.3)*	0.0	50.0	50.0
Inca berries	*1 (0.1)*	100.0	0.0	0.0
Caper berry	*1 (0.1)*	0.0	100.0	0.0
Gooseberry	*1 (0.1)*	0.0	100.0	0.0
Myrica Rubra	*1 (0.1)*	0.0	100.0	0.0
Grape	49 (6.5)	30.4	45.7	23.9
Orange	46 (6.1)	24.4	61.0	14.6
Apricot	44 (5.8)	50.0	34.1	15.9
Raspberry	33 (4.4)	50.0	33.3	16.7
Mandarin	24 (3.2)	27.3	68.2	4.5

Table S1.1 Frequency and severity of reported symptoms to fruit from most to least common in 757 patients (*continued*)

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Fig	24 (3.2)	36.4	31.8	31.8
Date	11 (1.5)	50.0	40.0	10.0
Jackfruit	11 (1.5)	27.3	27.3	45.5
Pomegranate	9 (1.2)	44.4	22.2	33.3
Citron	7 (0.9)	28.6	71.4	0.0
Passion fruit	4 (0.5)	33.3	66.7	0.0
Grapefruit	3 (0.4)	66.7	33.3	0.0
Persimmon	3 (0.4)	33.3	33.3	33.3
Lychee	3 (0.4)	0.0	33.3	66.7
Papaya	2 (0.3)	0.0	50.0	50.0
Lemon	1 (0.1)	0.0	100.0	0.0

Patients could report symptoms for more than one fruit. Therefore, the total number of patients does not equal the sum of patients with symptoms for each fruit.
For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Table S1.2 Frequency and severity of reported symptoms to vegetables from most to least common in 369 patients

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Tomato	**164 (44.4)**	**37.0**	**50.7**	**12.3**
Carrot	**130 (35.2)**	**45.6**	**35.2**	**19.2**
Paprika	64 (17.3)	29.3	53.4	17.2
Avocado	57 (15.4)	40.0	49.1	10.9
Potato	36 (9.8)	24.2	54.5	21.2
Leafy vegetable	31 (8.4)	18.4	52.6	28.9
Arugula	9 (2.4)	20.0	70.0	10.0
Lettuce	9 (2.4)	0.0	28.6	71.4
Iceberg lettuce	5 (1.4)	25.0	50.0	25.0
Chicory	4 (1.1)	0.0	50.0	50.0
Endive	1 (0.3)	0.0	0.0	100.0
Oak leaf lettuce	1 (0.3)	100.0	0.0	0.0
Lamb's lettuce	1 (0.3)	100.0	0.0	0.0
Cabbage total	29 (7.9)	27.6	55.2	17.2
Cabbage	8 (2.2)	0.0	75.0	25.0
Radish	7 (1.9)	42.9	28.6	28.6
Broccoli	4 (1.1)	0.0	100.0	0.0
Cauliflower	2 (0.5)	50.0	0.0	50.0
Kale	2 (0.5)	50.0	50.0	0.0

Table S1.2 Frequency and severity of reported symptoms to vegetables from most to least common in 369 patients (*continued*)

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Red cabbage	2 (0.5)	50.0	50.0	0.0
Turnip cabbage	1 (0.3)	100.0	0.0	0.0
Turnip greens	1 (0.3)	100.0	0.0	0.0
Sprouts	1 (0.3)	0.0	100.0	0.0
Sauerkraut	1 (0.3)	0.0	100.0	0.0
Celery	27 (7.3)	36.0	48.0	16.0
Eggplant	20 (5.4)	35.3	47.1	17.6
Spinach	18 (4.9)	42.9	42.9	14.3
Cucumber	12 (3.3)	36.4	18.2	45.5
Garlic	6 (1.6)	16.7	83.3	0.0
Leek	6 (1.6)	0.0	83.3	16.7
Fennel	6 (1.6)	16.7	33.3	50.0
Zucchini	5 (1.4)	40.0	40.0	20.0
Bean sprouts	4 (1.1)	25.0	50.0	25.0
Mushroom	4 (1.1)	0.0	75.0	25.0
Chili pepper	4 (1.1)	0.0	100.0	0.0
Asparagus	3 (0.8)	0.0	33.3	66.7
Pickle	3 (0.8)	0.0	0.0	100.0
Beet	3 (0.8)	100.0	0.0	0.0
Corn	2 (0.5)	0.0	50.0	50.0
Parsnip	2 (0.5)	50.0	50.0	0.0
Rhubarb	2 (0.5)	0.0	50.0	50.0
Sweet potato	2 (0.5)	0.0	50.0	50.0
Caper	1 (0.3)	0.0	100.0	0.0

Patients could report symptoms for more than one vegetable. *Therefore, the total number of patients does not equal the sum of patients with symptoms for each* vegetable.
For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Table S1.3. Frequency and severity of reported symptoms to seeds or pits from most to least common in 156 patients

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Seeds	101 (64.7)	24.1	39.1	36.8
Sesame	77 (49.4)	25.8	36.4	37.9
Mustard	12 (7.7)	20.0	40.0	40.0
Poppy seed	11 (7.1)	20.0	60.0	20.0
Flax seed	1 (0.6)	0.0	0.0	100.0
Pits	100 (64.1)	23.6	33.7	42.7
Pine nut	58 (37.2)	18.9	30.2	50.9

Table S1.3. Frequency and severity of reported symptoms to seeds or pits from most to least common in 156 patients (*continued*)

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Sunflower seed	24 (15.4)	23.8	33.3	42.9
Pumpkin seed	18 (11.5)	40.0	46.7	13.3

Patients could report symptoms for more than one seed or pit. Therefore, the total number of patients does not equal the sum of patients with symptoms for each seed or pit.
For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Table S1.4. Frequency and severity of reported symptoms to dairy from most to least common in 149 patients

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Cow's milk	145 (97.3)	9.5	75.9	14.6
Goat dairy	4 (2.7)	0.0	50.0	50.0
Sheep dairy	1 (0.7)	0.0	0.0	100.0

Patients could report symptoms for more than one dairy product. Therefore, the total number of patients does not equal the sum of patients with symptoms for each dairy product.
For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Table S1.5. Frequency and severity of reported symptoms to hen's egg from most to least common in 84 patients

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Hen's egg	84 (100)	7.0	71.8	21.1

For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Table S1.6. Frequency and severity of reported symptoms to crustaceans from most to least common in 82 patients

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Shrimp	79 (7.3)	22.9	44.3	32.9
Lobster	15 (18.3)	7.1	57.1	35.7
Krab	14 (1.3)	15.4	61.5	23.1
Langoustine	2 (0.2)	0.0	100.0	0.0

Patients could report symptoms for more than one crustacean. *Therefore, the total number of patients does not equal the sum of patients with symptoms for each* crustacean.
For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Table S1.7. Frequency and severity of reported symptoms to fish from most to least common in 74 patients

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
White fish total	51 (68.9)	19.6	39.1	41.3
White fish unspecified	15 (20.3)	21.4	42.9	35.7
Herring	8 (10.8)	14.3	28.6	57.1
Mackerel	5 (6.8)	25.0	0.0	75.0
Eel	5 (6.8)	0.0	100.0	0.0
Trout	3 (4.1)	33.3	33.3	33.3
Pangasius	2 (2.7)	50.0	0.0	50.0
Sardine	2 (2.7)	0.0	0.0	100.0
Plaice	2 (2.7)	50.0	50.0	0.0
Sole	2 (2.7)	0.0	50.0	50.0
Hake	1 (1.4)	0.0	100.0	0.0
Red snapper	1 (1.4)	0.0	100.0	0.0
Redfish	1 (1.4)	0.0	100.0	0.0
Slip tongue	1 (1.4)	0.0	0.0	100.0
Tilapia	1 (1.4)	100.0	0.0	0.0
Sea bass	1 (1.4)	0.0	100.0	0.0
Swordfish	1 (1.4)	0.0	0.0	100.0
Salmon	21 (28.4)	16.7	44.4	38.9
Tuna	18 (24.3)	12.5	56.3	31.3
Codfish	12 (16.2)	0.0	66.7	33.3
Fish unspecified	12 (16.2)	12.5	37.5	50.0

Patients could report symptoms for more than one fish. Therefore, the total number of patients does not equal the sum of patients with symptoms for each fish.
For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Table S1.8. Frequency and severity of reported symptoms to grains from most to least common in 63 patients

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Wheat	46 (73)	0.0	85.7	14.3
Rice	7 (11.1)	0.0	57.1	42.9
Rye	6 (9.5)	0.0	66.7	33.3
Oat merged	5 (7.9)	0.0	75.0	25.0
Buckwheat	3 (4.8)	0.0	33.3	66.7
Spells	2 (3.2)	0.0	100.0	0.0

Patients could report symptoms for more than one grain. Therefore, the total number of patients does not equal the sum of patients with symptoms for each grain.
For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Table S1.9. Frequency and severity of reported symptoms to mollusks from most to least common in 42 patients

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Bivalvia total	34 (100)	8.6	68.6	22.9
Mussel	22 (64.7)	10.5	73.7	15.8
Scallop	9 (26.5)	12.5	62.5	25.0
Oyster	7 (20.6)	0.0	40.0	60.0
Clams	2 (5.9)	0.0	100.0	0.0
Vongole	2 (5.9)	0.0	100.0	0.0
Squid	8 (100)	37.5	37.5	25.0

For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Table S1.10. Frequency and severity of reported symptoms to spices or herbs from most to least common in 37 patients

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Spices	32 (86.5)	12.5	62.5	25.0
Pepper	13 (35.1)	0.0	77.8	22.2
Ginger	4 (10.8)	0.0	50.0	50.0
Cinnamon	4 (10.8)	0.0	50.0	50.0
Turmeric	2 (5.4)	50.0	0.0	50.0
Paprika powder	2 (5.4)	0.0	100.0	0.0
Truffle	2 (5.4)	0.0	50.0	50.0
Anise	1 (2.7)	100.0	0.0	0.0
Clove	1 (2.7)	0.0	100.0	0.0
Nutmeg	1 (2.7)	0.0	100.0	0.0
Saffron	1 (2.7)	0.0	100.0	0.0
Liquorice	1 (2.7)	100.0	0.0	0.0
Herbs	15 (40.5)	15.4	53.8	30.8
Parsley	4 (10.8)	25.0	25.0	50.0
Basil	3 (8.1)	33.3	66.7	0.0
Cilantro	3 (8.1)	0.0	100.0	0.0
Rosemary	2 (5.4)	0.0	0.0	100.0
Thyme	2 (5.4)	0.0	0.0	100.0
Mint	1 (2.7)	0.0	100.0	0.0

Patients could report symptoms for more than one spice or herb. Therefore, the total number of patients does not equal the sum of patients with symptoms for each spice or herb.
For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Table S1.11. Frequency and severity of reported symptoms to meat from most to least common in 18 patients

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Red meat	14 (77.8)	25.0	41.7	33.3
Chicken	7 (38.9)	28.6	57.1	14.3

Patients could report symptoms for more than one meat product. Therefore, the total number of patients does not equal the sum of patients with symptoms for each meat product.
For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Table S1.X12. Frequency and severity of reported symptoms to insects from most to least common in 1 patients

Food	Frequency	Mild (%)	Moderate (%)	Severe (%)
Cricket	1 (100)	0	0	100

For the severity score, only patients with a known severity score were included
Color legend: Number of patients that reported mild, moderate or severe symptoms: ○ 0.0%; ● 100.0%

Table S2.1. Frequency of reported symptoms to seeds or pits with co-allergies to other seeds or pits

When symptoms to …

		% of patients with also symptoms to						
	N	**Sesame**	**Pine nut**	**Sunflower seed**	**Pumpkin seed**	**Mustard**	**Poppy seed**	**Flax seed**
Sesame	77		20.8	13.0	6.5	0.0	6.5	0.0
Pine nut	58	27.6		15.5	5.2	0.0	5.2	0.0
Sunflower seed	24	41.7	37.5		29.2	0.0	0.0	0.0
Pumpkin seed	18	27.8	16.7	38.9		0.0	0.0	0.0
Mustard	12	0.0	0.0	0.0	0.0		0.0	8.3
Poppy seed	11	45.5	27.3	0.0	0.0	0.0		0.0
Flax seed	1	0.0	0.0	0.0	0.0	100.0	0.0	

Color legend: ○ 0.0% co-allergies; ● 100.0% co-allergies

Table S2.2 Frequency of reported symptoms to tree nuts and peanut with co-allergies to other tree nuts and peanut

When symptoms to …		% of patients with also symptoms to												
	N	Hazelnut	Walnut	Almond	Cashew nut	Pecan nut	Pistachio	Macadamia	Brazil nut	Coconut	Chestnut	Beech nut	Kemiri nut	Peanut
Hazelnut	359		57.7	44.3	23.4	19.5	10.6	15.9	16.4	1.1	0.8	0.6	0.0	30.1
Walnut	325	63.7		39.1	24.6	24.3	11.1	14.5	15.1	0.6	0.9	0.6	0.0	29.5
Almond	221	71.9	57.5		30.3	24.9	13.1	18.1	18.1	1.8	0.9	0.9	0.0	39.8
Cashew nut	144	58.3	55.6	46.5		24.3	30.6	20.8	17.4	0.0	0.0	0.0	0.7	50.0
Pecan nut	94	74.5	84.0	58.5	37.2		18.1	31.9	37.2	0.0	1.1	1.1	0.0	33.0
Pistachio	72	52.8	50.0	40.3	61.1	23.6		30.6	22.2	1.4	0.0	0.0	0.0	52.8
Macadamia	70	81.4	67.1	57.1	42.9	42.9	31.4		37.1	1.4	0.0	0.0	0.0	42.9
Brazil nut	66	89.4	74.2	60.6	37.9	53.0	24.2	39.4		0.0	1.5	0.0	0.0	37.9
Coconut	11	36.4	18.2	36.4	0.0	0.0	9.1	9.1	0.0		0.0	0.0	0.0	27.3
Chestnut	4	75.0	75.0	50.0	0.0	25.0	0.0	0.0	25.0	0.0		25.0	0.0	25.0
Beech nut	2	100.0	100.0	100.0	0.0	50.0	0.0	0.0	0.0	0.0	50.0		0.0	0.0
Kemiri nut	1	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0
Peanut	317	34.1	30.3	27.8	22.7	9.8	12.0	9.5	7.9	0.9	0.3	0.0	0.0	

Color legend: ○ 0.0% co-allergies; ● 100.0% co-allergies

Table S2.3. Frequency of reported symptoms to fruit with co-allergies to other fruit

When symptoms to…	% of patients with also symptoms to																	
	N	Apple	Kiwi	Cherry	Peach	Pear	Strawberry	Pineapple	Banana	Nectarine	Plum	Mango	Melon	Grape	Orange	Apricot	Raspberry	Galia melon	Mandarin
Apple	482		45	43	37	40	26	15	16	20	14	10	7	7	8	8	6	3	4
Kiwi	374	58		29	26	30	24	20	19	17	11	11	8	9	7	7	5	4	4
Cherry	258	81	41		53	43	30	16	16	29	22	10	5	7	5	10	6	4	5
Peach	213	84	46	65		50	31	16	15	41	27	13	7	9	6	17	7	3	4
Pear	207	94	54	54	51		30	18	21	32	21	12	7	10	6	13	8	5	3
Strawberry	171	73	53	46	39	37		20	19	23	15	11	11	12	11	6	12	4	8
Pineapple	127	56	58	33	27	29	27		15	17	8	10	7	11	6	10	6	4	6
Banana	123	63	57	34	26	35	26	15		14	10	15	11	15	8	2	7	7	5
Nectarine	123	80	52	60	71	54	32	17	14		29	11	7	11	9	16	7	4	4
Plum	80	84	51	70	71	55	33	13	15	45		16	5	18	15	21	6	4	6
Mango	62	74	65	40	44	39	29	21	29	21	21		16	16	10	11	11	8	5
Melon	53	62	57	23	28	26	34	17	25	17	8	19		11	9	2	2	0	0
Grape	49	71	67	37	39	43	41	29	39	27	29	20	12		14	14	12	8	6
Orange	46	80	57	30	28	28	39	15	22	24	26	13	11	15		11	11	4	22
Apricot	44	84	57	61	82	59	23	30	5	45	39	16	2	16	11		9	9	0
Raspberry	33	85	55	45	45	48	61	21	24	24	15	21	3	18	15	12		3	9
Galia melon	24	63	67	46	25	42	25	21	38	21	13	21	0	17	8	17	4		4
Mandarin	24	75	58	50	33	29	54	29	25	21	21	13	0	13	42	0	13	4	
Fig	24	79	63	50	58	50	21	13	13	13	38	13	0	25	13	38	8	4	0
Watermelon	13	62	38	38	8	23	31	46	15	0	8	8	0	8	23	0	0	8	15
Date	11	82	36	36	27	27	27	18	18	18	27	18	0	36	9	18	18	0	0
Jackfruit	11	91	36	45	27	18	27	0	0	9	27	36	18	9	0	18	9	0	9
Pomegranate	9	67	78	56	44	33	11	22	22	44	11	22	0	22	0	0	0	11	0
Berries unspecified	8	50	25	38	38	13	50	0	13	38	0	13	13	38	0	13	13	13	0
Citron	7	71	29	29	43	71	71	0	29	29	0	29	0	0	43	0	14	0	14
Blueberry	7	71	71	29	43	71	29	43	29	43	43	14	0	14	29	0	29	29	29
Bilberry	7	57	29	29	43	14	43	0	29	14	0	0	0	0	29	0	29	0	14
Blackberry	7	86	43	43	43	43	57	14	43	29	0	29	0	14	14	0	29	14	14
Current	6	50	50	0	25	50	50	25	25	50	0	25	0	0	25	0	50	0	0
Black currant	5	60	0	60	0	0	40	20	0	0	0	0	20	0	20	0	40	0	0
Passion fruit	4	25	75	25	0	25	25	25	0	25	0	50	25	0	0	0	0	0	0
Honeydew melon	3	67	33	67	67	67	67	67	33	33	0	0	0	33	33	33	0	33	0
Grapefruit	3	100	100	33	33	33	33	67	0	33	33	0	0	0	33	0	0	0	33

Fig	Watermelon	Date	Jackfruit	Pomegranate	Berries unspecified	Citron	Blueberry	Billberry	Blackberry	Current	Black currant	Passion fruit	Honeydew melon	Grapefruit	Persimmon	Lychee	Goji berry	Papaya	Cantaloupe	Mulberry	Elderberry	Lemon	Inca berries	Caper berry	Gooseberry	Myrica rubra
4	2	2	2	1	1	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
4	1	1	1	2	1	1	1	1	1	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0
5	2	2	2	2	1	1	1	1	1	0	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0
7	0	1	1	2	1	1	1	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
6	1	1	1	1	0	2	2	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
3	2	2	2	1	2	3	1	2	2	1	1	1	1	1	0	1	0	0	1	0	1	0	0	0	0	0
2	5	2	0	2	0	0	2	0	1	1	1	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0
2	2	2	0	2	1	2	2	2	2	1	0	0	1	0	1	1	0	1	1	0	1	0	0	0	0	0
2	0	2	1	3	2	2	2	1	2	2	0	1	1	1	0	0	0	1	0	1	1	0	0	0	0	0
11	1	4	4	1	0	0	4	0	0	0	0	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0
5	2	3	6	3	2	3	2	0	3	2	0	3	0	0	0	0	0	2	0	0	0	0	0	0	0	0
0	0	0	4	0	2	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	2	8	2	4	6	0	2	0	2	0	0	0	2	0	0	2	0	2	0	0	2	0	0	0	0	0
7	7	2	0	0	0	7	4	4	2	2	2	0	2	2	0	2	0	0	2	0	0	0	0	0	0	0
20	0	5	5	0	2	0	0	0	0	0	0	0	2	0	0	0	0	2	2	0	2	0	0	0	0	0
6	0	6	3	0	3	3	6	6	6	6	6	0	0	0	0	3	0	3	0	0	6	0	0	0	0	0
4	4	0	0	4	4	0	8	0	4	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0	0	0
0	8	0	4	0	0	4	8	4	4	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0
	4	17	4	0	4	0	0	0	4	0	0	0	0	0	0	0	0	4	4	0	4	0	0	0	0	4
8		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0
36	0		0	9	0	0	0	0	9	0	0	0	0	0	0	0	0	9	0	0	9	0	0	0	0	0
9	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	11	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0
13	0	0	0	0		0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0
0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0		29	14	29	0	14	0	0	0	0	0	0	0	14	0	0	0	0	0
14	0	14	0	0	14	0	0	29		14	14	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0
0	0	0	0	0	0	0	0	25	25		25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	40	20	20		0	0	0	0	0	0	0	0	0	20	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	33	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0

Table S2.3. Frequency of reported symptoms to fruit with co-allergies to other fruit (*continued*)

When symptoms to…	% of patients with also symptoms to																	
	N	Apple	Kiwi	Cherry	Peach	Pear	Strawberry	Pineapple	Banana	Nectarine	Plum	Mango	Melon	Grape	Orange	Apricot	Raspberry	Galia melon	Mandarin
Persimmon	3	67	0	33	67	0	0	0	33	0	0	0	0	0	0	0	0	0	0
Lychee	3	67	33	33	33	0	33	0	33	0	0	0	0	33	33	0	33	0	0
Goji berry	3	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Papaya	2	50	0	50	50	0	0	0	50	50	50	50	0	50	0	50	50	0	0
Cantaloupe	2	100	100	100	0	50	50	0	50	0	50	0	0	0	50	50	0	50	0
Mulberry	2	50	50	50	50	50	0	0	0	50	0	0	0	0	0	0	0	0	0
Elderberry	2	100	0	100	50	0	50	0	50	50	50	0	0	50	0	50	100	0	0
Lemon	1	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0
Inca berries	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caper berry	1	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gooseberry	1	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrica rubra	1	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0

Color legend: ○ 0.0% co-allergies; ● 100.0% co-allergies

	Fig	Watermelon	Date	Jackfruit	Pomegranate	Berries unspecified	Citron	Blueberry	Bilberry	Blackberry	Current	Black currant	Passion fruit	Honeydew melon	Grapefruit	Persimmon	Lychee	Goji berry	Papaya	Cantaloupe	Mulberry	Elderberry	Lemon	Inca berries	Caper berry	Gooseberry	Myrica rubra
Fig	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		33	0	0	0	0	0	0	0	0	0	0
Watermelon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33		0	0	0	0	0	0	0	0	0	0
Date	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	33	0	0	0
Jackfruit	50	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	50	0	0	0	0	0
Pomegranate	50	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0
Berries unspecified	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0
Citron	50	0	50	0	0	0	0	0	50	50	0	50	0	0	0	0	0	0	50	0	0		0	0	0	0	0
Blueberry	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0
Bilberry	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0		0	0	0
Blackberry	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0
Current	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0
Black currant	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Table S2.4. Frequency of reported symptoms to vegetables with co-allergies to other vegetables

When symptoms to …	% of patients with also symptoms to																	
	N	Tomato	Carrot	Paprika	Avocado	Potato	Celery	Eggplant	Spinach	Cucumber	Lettuce	Arugula	Cabbage	Radish	Garlic	Leek	Fennel	Zucchini	Iceberg lettuce
Tomato	164		29	25	7	10	7	5	6	4	4	1	2	1	1	1	1	2	2
Carrot	130	37		22	7	12	11	2	2	3	4	4	1	2	0	2	1	2	2
Paprika	64	64	45		5	20	9	9	2	5	3	3	2	0	0	3	0	5	2
Avocado	58	19	16	5		3	3	7	3	3	2	0	0	2	0	0	0	0	2
Potato	36	47	42	36	6		17	11	8	0	6	3	6	6	0	6	0	3	3
Celery	27	44	52	22	7	22		7	4	4	4	4	0	7	0	11	4	4	0
Eggplant	20	45	10	30	20	20	10		5	0	0	0	0	0	0	5	0	10	0
Spinach	18	56	11	6	11	17	6	6		0	6	6	6	6	0	0	6	0	0
Cucumber	12	58	33	25	17	0	8	0	0		25	8	0	0	0	8	0	0	8
Lettuce	9	67	56	22	11	22	11	0	11	33		11	22	11	0	11	0	0	0
Arugula	9	22	56	22	0	11	11	0	11	11	11		0	11	0	0	0	0	11
Cabbage	8	50	13	13	0	25	0	0	13	0	25	0		13	13	13	0	0	0
Radish	7	29	29	0	14	29	29	0	14	0	14	14	14		0	0	14	0	0
Garlic	6	33	0	0	0	0	0	0	0	0	0	0	17	0		0	0	0	0
Leek	6	33	33	33	0	33	50	17	0	17	17	0	17	0	0		0	0	0
Fennel	6	17	17	0	0	0	17	0	17	0	0	0	0	17	0	0		0	0
Zucchini	5	60	60	60	0	20	20	40	0	0	0	0	0	0	0	0	0		0
Iceberg lettuce	5	60	60	20	20	20	0	0	0	20	0	20	0	0	0	0	0	0	
Bean sprouts	4	25	50	0	25	50	25	25	0	0	25	0	0	25	0	0	0	0	0
Chili pepper	4	0	0	25	25	0	0	0	0	0	0	0	0	0	25	0	0	0	0
Chicory	4	50	0	0	0	0	0	0	25	0	25	0	25	0	0	0	0	0	25
Broccoli	4	50	25	25	0	25	0	0	0	0	0	0	0	0	0	0	0	0	0
Mushroom	4	0	25	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0
Asparagus	3	33	0	0	0	0	0	0	0	0	33	0	33	0	0	0	0	0	0
Pickle	3	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Beet	3	33	33	33	0	0	33	0	67	0	0	33	0	33	0	0	33	0	0
Corn	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parsnip	2	50	100	50	0	50	100	0	0	0	0	0	0	0	0	0	0	0	0
Rhubarb	2	0	50	50	50	50	50	0	0	0	0	50	0	0	0	0	0	0	50
Sweet potato	2	50	50	100	0	50	0	0	0	50	0	50	0	0	0	0	0	0	0
Cauliflower	2	100	50	0	50	0	0	0	0	0	50	0	0	0	0	0	0	0	0
Kale	2	100	0	0	0	0	50	0	50	0	0	50	0	50	0	0	0	0	0
Red cabbage	2	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caper	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	Bean sprouts	Chili pepper	Chicory	Broccoli	Mushroom	Asparagus	Pickle	Beet	Corn	Parsnip	Rhubarb	Sweet potato	Cauliflower	Kale	Red cabbage	Caper	Endive	Oak leaf lettuce	Lamb's lettuce	Turnip cabbage	Turnip greens	Sprouts	Sauerkraut
Bean sprouts	1	0	1	1	0	1	1	1	0	1	0	1	1	1	1	0	0	1	1	1	1	1	0
Chili pepper	2	0	0	1	1	0	0	1	0	2	1	1	1	0	0	0	0	1	1	0	0	0	0
Chicory	0	2	0	2	0	0	0	2	0	2	2	3	0	0	0	0	0	0	0	0	0	0	0
Broccoli	2	2	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0
Mushroom	6	0	0	3	0	0	0	0	0	3	3	3	0	0	0	0	0	3	3	0	0	0	0
Asparagus	4	0	0	0	0	0	0	4	0	7	4	0	0	4	0	0	0	0	0	4	4	0	0
Pickle	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Beet	0	0	6	0	0	0	0	11	0	0	0	0	0	6	0	0	0	0	0	6	6	0	0
Corn	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0
Parsnip	11	0	11	0	0	11	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0
Rhubarb	0	0	0	0	11	0	0	11	0	0	11	11	0	11	0	0	0	0	0	11	11	0	0
Sweet potato	0	0	13	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cauliflower	14	0	0	0	0	0	0	14	0	0	0	0	0	14	0	0	0	0	0	14	14	0	0
Kale	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Red cabbage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caper	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	20	0	0	0	0
Oak leaf lettuce	0	0	20	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0
Lamb's lettuce		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Turnip cabbage	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Turnip greens	0	0		0	0	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sprouts	0	0	0		0	0	0	0	0	0	0	0	0	25	25	0	0	0	0	0	0	25	0
Sauerkraut	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	33	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0		0	0	0	0	0	33	0	0	0	0	0	33	33	0	0
	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0
	0	0	0	50	0	0	0	50	0	0	0	0	0		50	0	0	0	0	50	50	50	0
	0	0	0	50	0	0	0	0	0	0	0	0	0	50		0	0	0	0	0	0	50	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0

Table S2.4. Frequency of reported symptoms to vegetables with co-allergies to other vegetables (*continued*)

When symptoms to …	% of patients with also symptoms to																	
	N	Tomato	Carrot	Paprika	Avocado	Potato	Celery	Eggplant	Spinach	Cucumber	Lettuce	Arugula	Cabbage	Radish	Garlic	Leek	Fennel	Zucchini	Iceberg lettuce
Endive	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oak leaf lettuce	1	100	100	0	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0
Lamb's lettuce	1	100	100	0	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0
Turnip cabbage	1	100	0	0	0	0	100	0	100	0	0	100	0	100	0	0	0	0	0
Turnip greens	1	100	0	0	0	0	100	0	100	0	0	100	0	100	0	0	0	0	0
Sprouts	1	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sauerkraut	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Color legend: ○ 0.0% co-allergies; ● 100.0% co-allergies

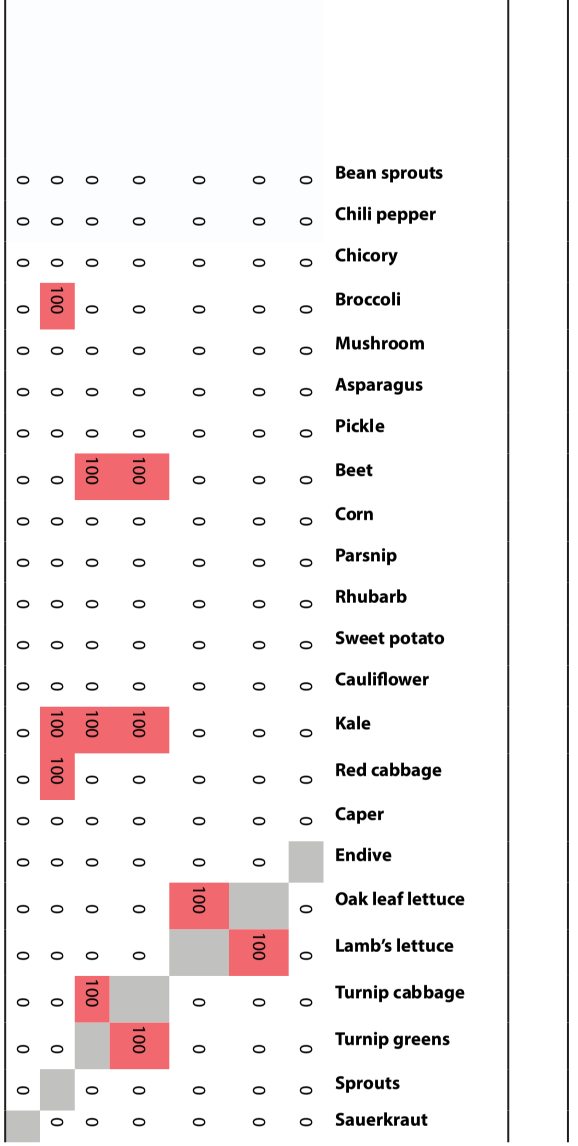

Bean sprouts
Chili pepper
Chicory
Broccoli
Mushroom
Asparagus
Pickle
Beet
Corn
Parsnip
Rhubarb
Sweet potato
Cauliflower
Kale
Red cabbage
Caper
Endive
Oak leaf lettuce
Lamb's lettuce
Turnip cabbage
Turnip greens
Sprouts
Sauerkraut

Table S3. Patient characteristics of patients with reported symptoms to at least plant foods

Total n = 291	**Pure sensitization groups**			**Combination of sensitization groups**				**No sens***
	PR-10 N=150	SS N=12	LTP N=3	PR-10/ SS N=53	PR-10/ LTP N=15	LTP/SS N=2	PR-10/ LTP/SS N=11	No sens* N=45
Highest severity score; n (%)**								
Mild	40 (26.7)	3 (25.0)	0	12 (22.6)	1 (6.7)	0	1 (9.1)	9 (20.0)
Moderate	61 (40.7)	3 (25.0)	1 (33.3)	22 (41.5)	11 (73.3)	0	5 (45.5)	20 (44.4)
Severe	49 (32.7)	6 (50.0)	2 (66.6)	19 (35.8)	3 (20.0)	2 (100)	5 (45.5)	16 (35.6)

*No sensitization to PR-10, SS and LTP

Abbreviations: *PR-10 = pathogenesis-related-10 protein, SS = seed storage protein, LTP = lipid transfer protein*

Table S4. Frequency and severity of reported symptoms to hazelnut, peanut, apple, and kiwi in patients sensitized to PR-10 protein and/or non-PR-10 protein of the culprit food

Severity	**Mono PR-10**		**Mono non-PR-10**		**Combination**		**Linear by linear**
Hazelnut (N=232)	*N=194*		*N=8*		*N=30*		0.025
Mild	76	(39.2)	3	(37.5)	5	(16.7)	
Moderate	61	(31.4)	1	(12.5)	10	(33.3)	
Severe	57	(29.4)	4	(50.0)	15	(50.0)	
Peanut (N=178)	*N=80*		*N=36*		*N=62*		0.001
Mild	23	(28.7)	1	(2.8)	3	(4.8)	
Moderate	28	(35.0)	16	(44.4)	32	(51.6)	
Severe	29	(36.3)	19	(52.8)	27	(43.5)	
Apple (N=56)	*N=45*		*N=5*		*N=6*		0.598
Mild	18	(40.0)	3	(60.0)	2	(33.3)	
Moderate	15	(33.3)	1	(20.0)	3	(50.0)	
Severe	12	(26.7)	1	(20.0)	1	(16.7)	
Kiwi (N=31)	*N=18*		*N=6*		*N=7*		1.000
Mild	6	(33.3)	2	(33.3)	1	(14.3)	
Moderate	7	(38.9)	3	(50.0)	3	(42.9)	
Severe	5	(27.8)	1	(16.7)	3	(42.9)	

Table S5. Comparison of the frequency of the food analyzed in the EuroPrevall study

Reported symptoms to	N (%) 2018-2023	EP N (%) 2006-2009	Rank	EP Rank
Apple	482 (44.4)	68 (52.3)	1	1
Kiwi	374 (34.5)	56 (43.8)	2	2
Hazelnut	359 (33.1)	41 (35.7)	3	4
Walnut	325 (30.0)	46 (40.4)	4	3
Peanut	317 (29.2)	37 (30.1)	5	5
Cherry	258 (23.8)	35 (26.9)	6	8
Almond	221 (20.4)	34 (26.2)	7	9
Peach	213 (19.6)	37 (29.6)	8	6
Pear	207 (19.1)	38 (29.2)	9	7
Cow's milk	174 (16.0)	9 (7.1)	10	21
Strawberry	171 (15.8)	19 (14.6)	11	14
Tomato	164 (15.1)	21 (16.5)	12	12
Cashew nut	144 (13.3)	25 (19.2)	13	11
Carrot	130 (12.0)	15 (11.9)	14	15
Pineapple	127 (11.7)	13 (10)	15	17
Banana	123 (11.3)	20 (15.6)	16	13
Nectarine	123 (11.3)	28 (21.5)	16	10
Pecan nut	94 (8.7)	3 (2.3)	17	30
Hen's egg	84 (7.7)	10 (7.9)	18	19
Plum	80 (7.4)	14 (10.8)	19	16
Shrimp	79 (7.3)	11 (9.2)	20	18
Sesame	77 (7.1)	9 (7.0)	21	22
Pistachio	72 (6.6)	10 (7.7)	22	20
Macadamia nut	70 (6.5)	1 (0.8)	23	33
Brazil nut	66 (6.1)	4 (3.1)	24	28
Mango	62 (5.7)	3 (2.3)	25	30
Pine nut	58 (5.3)	3 (2.3)	26	30
Avocado	57 (5.3)	1 (0.8)	26	33
Grape	49 (4.5)	6 (4.6)	27	25
Wheat	46 (4.2)	1 (0.8)	28	33
Apricot	44 (4.1)	12 (9.2)	29	18
Potato	36 (3.3)	3 (2.3)	30	30
Celery	27 (2.5)	1 (0.8)	31	33
Fig	24 (2.2)	1 (0.8)	32	33
Green pea	23 (2.1)	3 (2.3)	33	30
Mussel	22 (2.0)	3 (2.3)	34	30
Lentils	20 (1.8)	3 (2.5)	35	29
Spinach	18 (1.7)	4 (3.1)	36	28

Table S5. Comparison of the frequency of the food analyzed in the EuroPrevall study (*continued*)

Reported symptoms to	N (%) 2018-2023	EP N (%) 2006-2009	Rank	EP Rank
Pumpkin seed	18 (1.7)	1 (0.8)	36	33
Tuna	18 (1.7)	2 (1.5)	36	32
Lobster	15 (1.4)	2 (1.5)	37	32
Chickpea	14 (1.3)	2 (1.5)	38	32
Soybean	14 (1.3)	3 (2.5)	38	29
Krab	14 (1.3)	3 (2.3)	38	30
Pepper	13 (1.2)	7 (5.4)	39	24
Cucumber	12 (1.1)	8 (6.2)	40	23
Mustard	12 (1.1)	7 (5.4)	40	24
Coconut	11 (1.0)	4 (3.1)	41	28
Poppy seed	11 (1.0)	4 (3.2)	41	27
Lettuce	9 (0.8)	2 (1.5)	42	32
Herring	8 (0.7)	1 (0.8)	43	33
Blueberry	7 (0.6)	1 (0.8)	44	33
Oyster	7 (0.6)	1 (0.8)	44	33
Chicken	7 (0.6)	2 (1.5)	44	32
Leek	6 (0.6)	1 (0.8)	44	33
Eel	5 (0.5)	1 (0.8)	45	33
Passion fruit	4 (0.4)	5 (3.8)	46	26
Grapefruit	3 (0.3)	1 (0.8)	47	33
Lychee	3 (0.3)	1 (0.8)	47	33
Buckwheat	3 (0.3)	0 (0.0)	47	34
Basil	3 (0.3)	1 (0.8)	47	33
Corn	2 (0.2)	2 (1.6)	48	31
Cauliflower	2 (0.2)	1 (0.8)	48	33
Langoustine	2 (0.2)	1 (0.8)	48	33
Lemon	1 (0.1)	1 (0.8)	49	33

Abbreviations: EP = EuroPrevall

For the severity score, only patients with a known severity score were included.

Color legend, columns:

Color legend:

- Food: Crustaceans; meat; mollusks; fish; nuts; seed or pits; grains; insects; fruit; vegetables; legumes; spices and herbs; dairy; hen's egg
- N (%) or EP N (%): Colored if the percentage is at least twice as high as the other group, > 10 patients and < 10 patients in one of the studies

3

A European-Japanese study on peach allergy: IgE to Pru p 7 associates with severity

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* Shared first authorship, ■ Shared last authorship
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Introduction

Pru p 3 and Pru p 7 have been implicated as risk factors for severe peach allergy. This study aimed to establish sensitization patterns to five peach components across Europe and in Japan, to explore their relation to pollen and foods and to predict symptom severity.

Methods

In twelve European (EuroPrevall project) and one Japanese outpatient clinic, a standardized clinical evaluation was conducted in 1231 patients who reported symptoms to peach and/or were sensitized to peach. Specific IgE against Pru p 1, 2, 3, 4 and 7 and against Cup s 7 was measured in 474 of them. Univariable and multivariable Lasso regression was applied to identify combinations of parameters predicting severity.

Results

Sensitization to Pru p 3 dominated in Southern Europe but was also quite common in Northern and Central Europe. Sensitization to Pru p 7 was low and variable in the European centers but very dominant in Japan. Severity could be predicted by a model combining age of onset of peach allergy, probable mugwort, *Parietaria* pollen and latex allergy, and sensitization to Japanese cedar pollen, Pru p 4 and Pru p 7 which resulted in an AUC of 0.73 (95% CI 0.73-0.74). Pru p 3 tended to be a risk factor in South Europe only.

Conclusions

Pru p 7 was confirmed as a significant risk factor for severe peach allergy in Europe and Japan. Combining outcomes from clinical and demographic background with serology resulted in a model that could better predict severity than CRD alone.

INTRODUCTION

Peach is among the most prevalent plant food allergies in Europe and Japan.[1,2] The prevalence of sensitization in adults increased from 5.4% in 2010[3] to 7.9% in 2013[4]. In adults, the EuroPrevall study showed a mean prevalence of probable peach allergy (reported symptoms plus matching IgE-sensitization) of 0.80%.[5]

Component-resolved diagnostics (CRD) defines sensitization profiles of patients at the molecular level.[6] Five food allergen components from peach are listed in the IUIS/WHO allergen database: Pru p 1 (Bet v 1-like protein [PR-10]), Pru p 2 (thaumatin-like protein [TLP]), Pru p 3 (lipid transfer protein [LTP]), Pru p 4 (profilin) and Pru p 7 (gibberellin-regulated protein [GRP]).[7]

In Northern and Central Europe patients are dominantly sensitized to Pru p 1 and in Mediterranean countries to Pru p 3.[8–10] Sensitization to Pru p 4 is less regionally determined.[8,9,11] Recently, sensitization to Pru p 7 (peamaclein), a gibberellin-regulated protein (GRP) was reported[12]. It is thought to originate from primary sensitization to GRPs in pollen of *Cupressaceae* trees as cypress[13] and Japanese cedar[14].

CRD could help to better estimate the risk of severe reactions to peach.[15] In smaller studies in Southern Europe, Pru p 3 was a marker for severe peach allergy[16], and also Pru p 7 was associated with more severe reactions, both in Japan[1] and in Southern Europe[13]. It remains difficult to compare these results because study designs vary. The EuroPrevall project used study protocols across Europe with standardized case-record forms (CRFs), centralized serum testing and identical challenge protocols.[17] We added data from a clinical center from Japan where it was expected that sensitization to Pru p 7 is more common due to the importance of allergy to Japanese cedar pollen, using the EuroPrevall CRF.

Our aims were to (1) identify differences in sensitization pattern to peach components across Europe and Japan; (2) assess relationships between IgE to peach components and pollen and foods, providing insight into possible primary sensitizers; and (3) develop a model to predict severity of peach allergy.

METHODS

Study design, setting, and patients

Patients from twelve European centers and one Japanese center were included. The European patients were enrolled in the outpatient clinic survey of the EuroPrevall project[18] of which the methodology was published previously.[17] Patients from the EuroPrevall study were selected for this study if they had a reported adverse reaction within 2 hours of ingestion of peach and/or were sensitized to peach (positive IgE to peach extract/component, positive SPT or prick-to-prick test (PTP) to peach).

In Japan, patients were included if they reported adverse reactions within 2 hours of ingestion of peach and had an IgE to peach extract ≥ 0.10 kU_A/L. Patients with only oral allergy symptoms to peach were excluded.

To gain more insight into the molecular sensitization patterns of the sensitized patients with and without symptoms, in the European centers peach sensitized patients with and without symptoms were included and in the Japanese center this was only possible for patients with symptoms to peach.

Ethical approval and informed consent were obtained in each center and from each participating patient.

Data collection

In the EuroPrevall study, clinical data were collected using a detailed standardized CRF.[17] Skin prick tests were performed with peach extract (ALK, Madrid, Spain). Total IgE, specific IgE to latex, 12 inhalant allergen sources, peach and 23 other foods commonly implicated in food allergy across Europe were measured in serum (ImmunoCAP, ThermoFisher Scientific, Uppsala, Sweden).[17] Prick-to-prick testing (PTP) with fresh peach peel or pulp was performed in case of negative SPT with peach extract, as indicated by local practice. In addition, in the course of the present study, IgE against Japanese cedar (*Cryptomeria japonica)* pollen was determined for the EuroPrevall patients to allow comparison to the Japanese patients. In the CRD analysis, specific IgE against rPru p 1, rPru p 2, rPru p 3, rPru p 4, rPru p 7 and rCup s 7 (GRP protein of cypress) was measured. Due to restricted availability of the custom-made peach component ImmunoCAP tests (rPru p 2, rPru p 7 and rCup s 7) analysis was only performed in a selection of patients (37%) (for detailed description see **Supplemental S1**). A double-blind placebo-controlled food challenge (DBPCFC) for peach was performed in all consenting patients who reported symptoms except for patients with a history of severe life-threatening anaphylaxis.[17]

In Japan, the same standardized EuroPrevall CRF was retrospectively filled out. IgE to peach extract, Japanese cedar pollen and cypress pollen, and the same CRD analysis as for the European patients was performed.

The validated oFASS-3 scoring was used to classify the severity; oFASS-3 grade 1 (mild: isolated oropharyngeal symptoms), grade 2 (moderate: grade 1 symptoms and/or symptoms of the skin, eye, upper airways and/or digestive system) and grade 3 (severe: grade 1 and/or 2 symptoms and/or symptoms of the lower airways, cardiovascular and/or nervous system).[19]

Definitions

Probable peach allergy was defined as a combination of reported symptoms upon ingestion of peach and matching IgE sensitization (positive SPT, PTP, and/or IgE against peach and/or any components). SPT results were expressed as allergen/histamine wheal ratios. A wheal ratio ≥ 0.5 and IgE levels ≥ 0.35 kU_A/L were considered positive for both the EuroPrevall and Japanese's patients.

Probable allergy to inhalant allergen sources and latex was defined as reported symptoms and matching IgE sensitization in SPT and/or ImmunoCAP. Since allergic symptoms to cat and dog were collected as symptoms to epithelia and not as single outcomes, and symptoms to Japanese cedar pollen were only collected in Japan, sensitization to cat, dog and Japanese cedar pollen was used instead.

Statistical analyses

Patients' demographical characteristics, clinical history and symptom severity were described using means with standard deviation (SD) or medians with interquartile range (Q1, Q3) for respectively normally or non-normally distributed continuous variables and frequency and proportions for categorical variables. Proportions of patients reporting symptoms to peach and/or sensitized to peach, were explored overall and for each participating center separately. Differences between centers were tested using ANOVAs, Kruskal-Wallis tests, or chi-square tests, respectively.

Spearman rho coefficients were calculated to assess relationships between levels of IgE to peach components, and levels of IgE to foods, inhalants allergens and latex if at least 10 patients with both measured IgE for a peach component and for another extract were present.

For severity prediction, only patients with a probable peach allergy were analyzed. Peach allergy severity was dichotomized as mild to moderate (i.e. oFASS-3 grades 1/2) versus severe (i.e. oFASS-3 grade 3).

First univariable logistic regression analysis was performed followed by multivariable logistic regression using Lasso regression (R package 'glmnet'). Missing data was imputed as preprocessing step (**Supplemental S2**).

A 3-step approach to building prediction models was used for patients with probable peach allergy in the Japanese and European centers together and for European centers only. In step 1 (model 1), demographic, clinical history and inhalant allergy variables were entered as predictors. In step 2 (model 2) both the selected predictors of model 1, and IgE levels to peach extract, cypress pollen and Japanese cedar pollen, and the SPT with peach extract results were used as predictors. In step 3 (model 3) the predictors selected by model 2 and the 10 log IgE levels to peach components were used. A 4th separate model was built with only the peach components as predictors (**Supplemental S3**).

These models were subsequently applied to a subset of European patients that had undergone a DBPCFC together with those who had a history of severe anaphylaxis to peach (oFASS-3 grade 3). When the model fit was evaluated for the patients in this group, for those that underwent a challenge, challenge-reported symptoms were used, for those that reported anaphylaxis, self-reported symptoms were used. Overall, model predictive performance were evaluated using AUC-ROC and sensitivity/specificity with an optimal cut-off determined by the Youden-index.

Analyses were conducted with R version 4.2.1.

RESULTS

Population characteristics

In total 1231 patients were included in this study (**Table 1**), the mean age was 28.6 (±15.0) and 57.8% were female. There were a few significant differences between centers with respect to the age of patients at inclusion (younger in Prague being a pediatric clinic), age of onset of peach allergy (older in Milan) and severity of reported symptoms (more severe in Sagamihara as expected based on exclusion of oFASS-3 grade 1; **Supplemental S1**). Of the 1231 patients, 561 (45.6%) reported symptoms of which 477 (38.7%) were also sensitized, i.e., having probable peach allergy. Almost half of the 561 patients reported mild symptoms (46.9%) and around a quarter moderate or severe symptoms (26.9% and 26.2%, respectively). Most reported were symptoms of the oral cavity (85.4%), skin (33.2%), upper airways (27.8%), digestive system (13.9%) and lower airways (10.5%). Fewer patients reported neurologic symptoms (2.0%), life-threatening anaphylaxis or cardiovascular symptoms (3.0%). In total, 670 patients (54.4%) reported no symptoms of which all were sensitized. There were no patients without symptoms and not sensitized (**Supplemental S4**).

Table 1. Characteristics of patients with peach sensitization and/or peach symptoms

Characteristics	Total (N = 1231)	Zürich (N = 204)	Stras-bourg (N = 119)	Utrecht (N = 131)	Man-chester (N = 56)	Lodz (N = 107)	Prague (N = 55)
Age, mean (SD)	28.6 ± 15.0	33.1 ± 13.5	32.1 ± 13.1	29.9 ± 12.2	31.3 ± 15.4	29.4± 17.7	12.0 ± 11.1
Female sex	712 (57.8%)	123 (60.3%)	81 (68.1%)	83 (63.4%)	35 (62.5%)	70 (65.4%)	25 (45.5%)
Age onset, mean (SD) †	21.1 ± 14.0	22.9 ± 11.5	23.5 ± 10.6	21.2 ± 12.3	27.3 ± 16.4	25.0 ±17.6	13.8 ± 9.47
Severity oFASS-3							
No symptoms	670 (54.4%)	103 (50.5%)	45 (37.8%)	66 (50.4%)	30 (53.6%)	54 (50.5%)	47 (85.5%)
oFASS-3 grade 1	263 (21.4%)	52 (25.5%)	56 (47.1%)	32 (24.4%)	4 (7.1%)	11 (10.3%)	4 (7.3%)
oFASS-3 grade 2	151 (12.3%)	23 (11.3%)	5 (4.2%)	15 (11.5%)	6 (10.7%)	25 (23.4%)	2 (3.6%)
oFASS-3 grade 3	147 (11.9%)	26 (12.7%)	13 (10.9%)	18 (13.7%)	16 (28.6%)	17 (15.9%)	2 (3.6%)
Probable peach allergy	477 (38.7%)	86 (42.2%)	67 (56.3%)	63 (48.1%)	23 (41.1%)	26 (24.3%)	6 (10.9%)
Peach sensitisation ‡/§							
Positive SPT peach	269 (21.9%)	29 (14.2%)	7 (5.9%)	9 (6.9%)	7 (12.5%)	13 (12.1%)	13 (23.6%)
Positive IgE to peach	957 (77.7%)	151 (74.0%)	105 (88.2%)	73 (55.7%)	49 (87.5%)	74 (69.2%)	52 (94.5%)
CRD performed	474 (38.5%)	61 (29.9%)	32 (26.9%)	39 (29.8%)	22 (39.3%)	50 (46.7%)	20 (36.4%)
Positive Pru p 1	254 (53.6%)	51 (83.6%)	28 (87.5%)	34 (87.2%)	14 (63.6%)	42 (84.0%)	17 (85.0%)
Positive Pru p 2	20 (4.2%)	2 (3.3%)	1 (3.1%)	4 (10.3%)	1 (4.5%)	4 (8.0%)	0 (0%)
Positive Pru p 3	208 (43.9%)	22 (36.1%)	7 (21.9%)	12 (30.8%)	10 (45.5%)	14 (28.0%)	12 (60.0%)
Positive Pru p 4	94 (19.8%)	19 (31.1%)	4 (12.5%)	12 (30.8%)	6 (27.3%)	12 (24.0%)	3 (15.0%)
Positive Pru p 7	49 (10.3%)	3 (4.9%)	1 (3.1%)	6 (15.4%)	1 (4.5%)	5 (10.0%)	0 (0%)
Positive Cup s 7	36 (7.6%)	0 (0%)	0 (0%)	2 (5.1%)	0 (0%)	3 (6.0%)	0 (0%)

Table 1. Characteristics of patients with peach sensitization and/or peach symptoms (*continued*)

Characteristics	Vilnius (N = 133)	Sofia (N = 34)	Reykjavik (N = 32)	Milan (N = 95)	Madrid (N = 118)	Athens (N = 112)	Sagami-hara (N = 35)	P-value
Age, mean (SD)	27.8 ± 12.9	17.9 ± 12.7	30.1 ± 10.5	39.2 ± 14.7	21.4 ± 13.9	26.7 ± 11.6	19.9 ± 19.9	**<0.001**
Female sex	75 (56.4%)	15 (44.1%)	18 (56.3%)	59 (62.1%)	58 (49.2%)	51 (45.5%)	19 (54.3%)	**0.006**
Age onset, mean (SD) [†]	13.8 ± 8.04	11.8 ± 8.50	14.4 ± 10.1	27.9 ± 15.8	13.6 ± 10.2	12.9 ± 7.89	19.6 ± 20.0	**<0.001**
Severity oFASS-3								**<0.001**
No symptoms	116 (87.2%)	21 (61.8%)	27 (84.4%)	32 (33.7%)	56 (47.5%)	73 (65.2%)	0 (0%)	
oFASS-3 grade 1	9 (6.8%)	1 (2.9%)	0 (0%)	42 (44.2%)	35 (29.7%)	17 (15.2%)	0 (0%)	
oFASS-3 grade 2	5 (3.8%)	9 (26.5%)	4 (12.5%)	12 (12.6%)	16 (13.6%)	14 (12.5%)	15 (42.9%)	
oFASS-3 grade 3	3 (2.3%)	3 (8.8%)	1 (3.1%)	9 (9.5%)	11 (9.3%)	8 (7.1%)	20 (57.1%)	
Probable peach allergy	15 (11.3%)	5 (14.7%)	4 (12.5%)	56 (58.9%)	58 (49.2%)	36 (32.1%)	32 (91.4%)	**<0.001**
Peach sensitisation [‡]/[§]								
Positive SPT peach	18 (13.5%)	11 (32.4%)	8 (25.0%)	25 (26.3%)	46 (39.0%)	83 (74.1%)	0 (0%)	**<0.001**
Positive IgE to peach	122 (91.7%)	17 (50.0%)	22 (68.8%)	87 (91.6%)	86 (72.9%)	95 (84.8%)	24 (68.6%)	**<0.001**
CRD performed	32 (24.1%)	13 (38.2%)	20 (62.5%)	20 (21.1%)	60 (50.8%)	71 (63.4%)	34 (97.1%)	
Positive Pru p 1	26 (81.3%)	4 (30.8%)	9 (45.0%)	7 (35.0%)	7 (11.7%)	4 (5.6%)	11 (32.4%)	**<0.001**
Positive Pru p 2	2 (6.3%)	1 (7.7%)	1 (5.0%)	0 (0%)	2 (3.3%)	2 (2.8%)	0 (0%)	0.617
Positive Pru p 3	7 (21.9%)	4 (30.8%)	3 (15.0%)	13 (65.0%)	37 (61.7%)	62 (87.3%)	5 (14.7%)	**<0.001**
Positive Pru p 4	5 (15.6%)	4 (30.8%)	6 (30.0%)	2 (10.0%)	9 (15.0%)	5 (7.0%)	7 (20.6%)	**0.032**
Positive Pru p 7	2 (6.3%)	1 (7.7%)	0 (0%)	0 (0%)	7 (11.7%)	1 (1.4%)	22 (64.7%)	**<0.001**
Positive Cup s 7	1 (3.1%)	1 (7.7%)	0 (0%)	0 (0%)	6 (10.0%)	0 (0%)	23 (67.6%)	**<0.001**

All measurements are in N (%) unless otherwise specified. [†]Reported age of onset of symptoms against peach. [‡]The total number differs for the different centers and tests. Not all patients underwent all tests. The percentage given in brackets is the percentage of this variable total number that were tested. [§]The results show the number and percentage of patients with positive sensitization according to each test. SPT/PTP was considered positive if allergen/histamine wheal ratios ≥ 0.5; Im-

munoCAP if IgE levels ≥ 0.35 kU_A/L. *Abbreviations: CRD = component-resolved diagnostics, SPT = skin prick test, PTP = prick-to-prick test. P- values between centers were determined for exploratory purposes (no correction for multiple testing) using the Pearson χ^2 test for categorical variables and the ANOVA or Kruskall-Wallis test for continuous variables.*

Peach sensitization patterns across Europe and Japan

Of the 1231 patients, 1147 patients were sensitized to peach. The mean levels of sensitization were slightly higher in the group with self-reported symptoms compared to the group without symptoms. However, this did not reach significance (**Figure 1**).

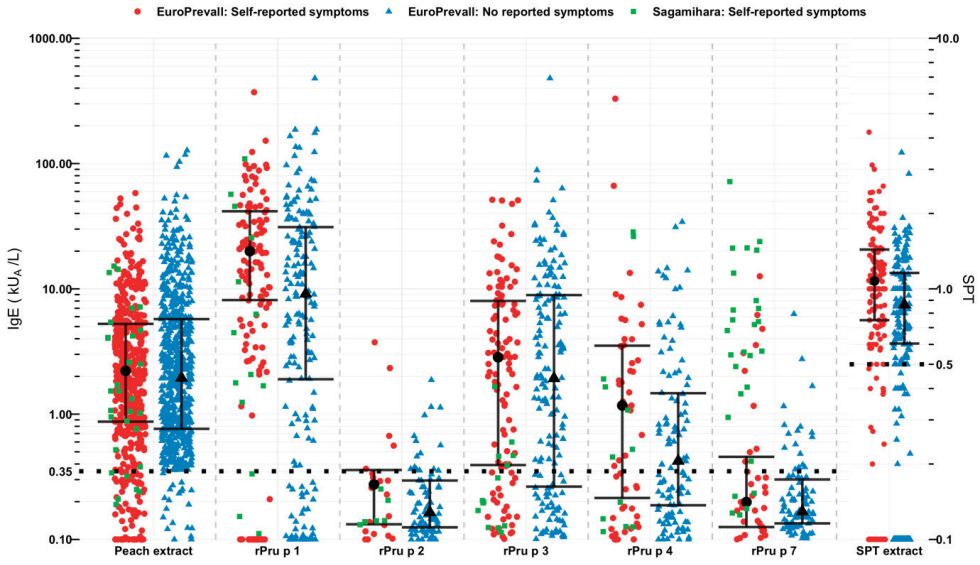


Figure 1. IgE to peach extract and peach allergens and size of skin prick test in patients with and without self-reported symptoms in twelve European centers and in patient with self-reported symptoms of Japan. The black symbols and error bars indicate the median IgE response and interquartile ranges of only the EuroPrevall data. *Abbreviations: SPT = skin prick test*

CRD was assessed in 474 sensitized patients with or without symptoms to peach (**Table 1; Figure 2).** There were significant differences between the 13 centers in the number of patients sensitized to Pru p 3 and to Pru p 7. In Athens 87.3% was sensitized to Pru p 3, in Sagamihara 14.7%. The percentage sensitized to Pru p 7 was 64.7% in Sagamihara and ranged in Europe from 15.4% in Utrecht to 0% in Prague, Reykjavik and Milan.

Amongst the European patients sensitized to peach, the most frequently recognized allergen was Pru p 1 (55.2%), followed by Pru p 3 (46.1%), Pru p 4 (19.8%), Pru p 7 (6.1%), and Pru p 2 (4.5%) (**Figure 2**). In the subgroup of patients with probable peach allergy (218/474) these percentages were similar (**Supplemental S5**). Sensitization to Pru p 1 was most common in Northern and Central Europe (Zürich, Strasbourg, Utrecht, Manchester, Lodz, Prague, and Vilnius). In the Mediterranean area (Madrid, Milan and Athens)

sensitization to Pru p 3 dominated (from 66.7% to 100%), but it was also quite common in Northern and Central Europe (from 13% to 60%). Sensitization to Pru p 7 was not often observed in patients from European centers (mostly < 10%). In all centers, except for Madrid, Athens and Reykjavik, sensitization to Pru p 4 was detected at clearly lower frequency than to Pru p 1 and at lower or similar frequency as to Pru p 3. Pru p 2 was hardly recognized in any of the twelve centers.

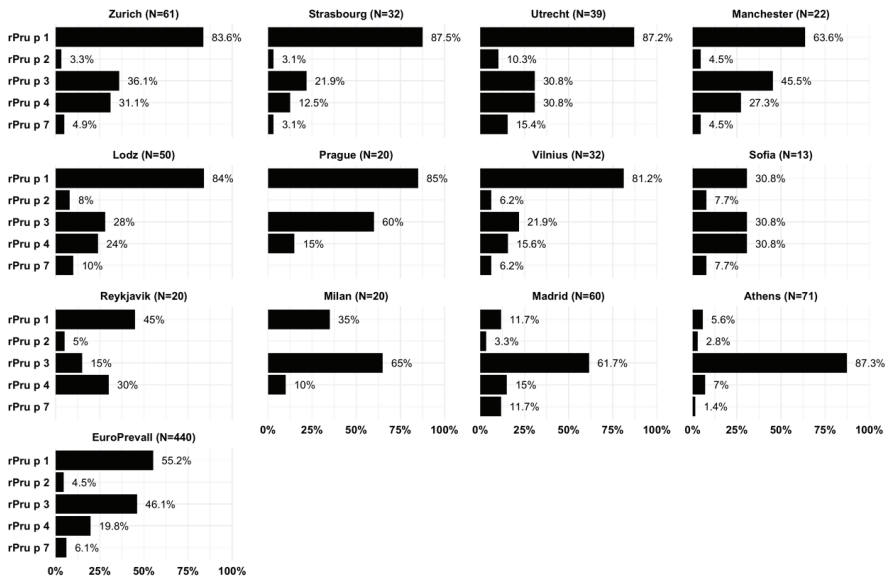


Figure 2. IgE to peach components (IgE levels ≥ 0.35 kU_A/L) across Europe amongst sensitized patients with or without reported symptoms.

Relationships between IgE to peach components and other allergens in Europe

Correlations between the level of IgE to peach components and to inhalants and foods are shown in **Figure 3** (overall) and **Supplemental S6** (per center). The strongest correlation overall was between IgE to Pru p 1 and to birch pollen (ρ = 0.9), with stronger associations (ρ > 0.9) in most centers with high birch pollen exposure (Northern/Central Europe, except Utrecht) compared to centers with low exposure (ρ≤0.8 in Madrid, Athens, and Reykjavik). IgE to Pru p 7 was moderately associated with pollen from all species except birch, but most strongly with IgE to Japanese cedar and cypress pollen (both ρ=0.7). IgE to profilin (Pru p 4) was associated with all pollen species except birch pollen. IgE to Pru p 3 was hardly associated with sensitization to pollen.

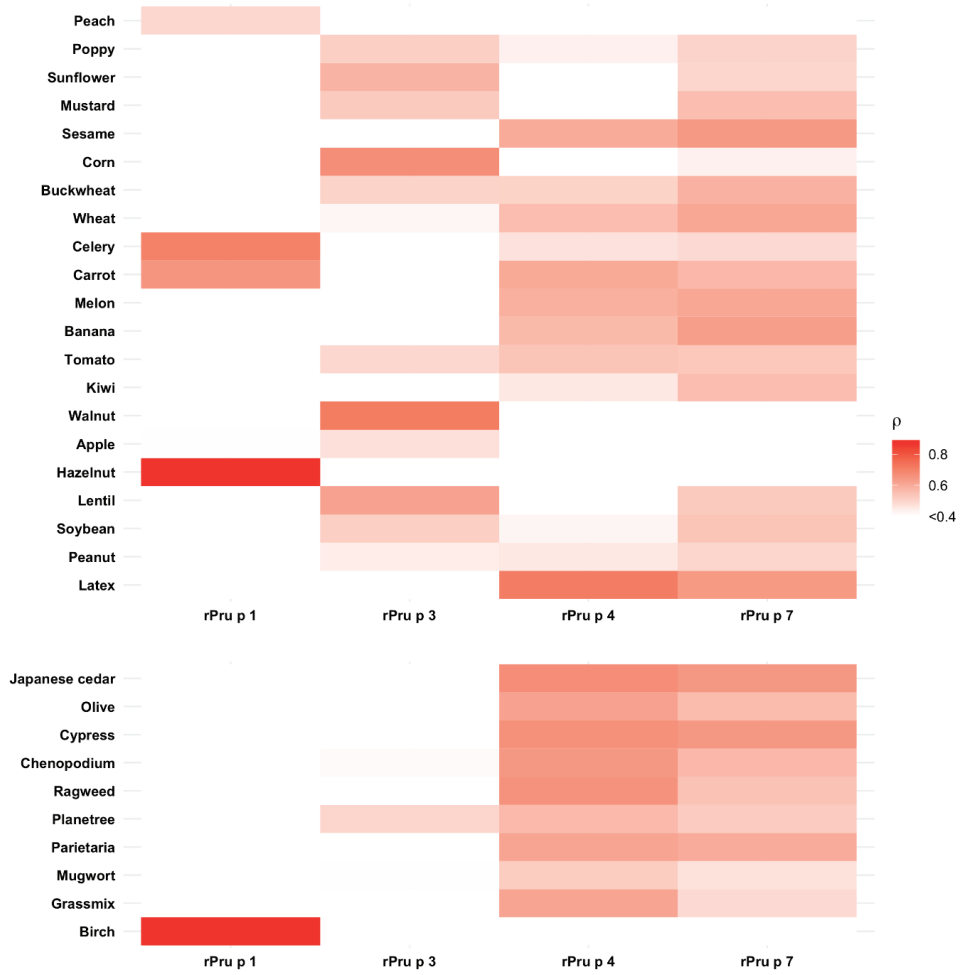


Figure 3. Correlation between IgE to peach components and IgE to pollen and foods other than peach across Europe

Regarding the correlations between IgE to peach components and foods, the strongest correlation, overall and in most centers, was seen between IgE to Pru p 1 and hazelnut (**Figure 3** and **Supplemental S6**). IgE to Pru p 3 showed moderate associations with food specific IgE, the highest with walnut and only weakly with apple and peach. Only in Madrid and Athens, strong correlations were found with IgE to apple and peach ($\rho = 0.9$). For IgE responses against Pru p 4 and Pru p 7, overall, a broad pattern of associations was observed, with no food clearly standing out.

Predictors for severity of peach allergy in Europe and Japan

Of the patients with probable peach allergy (N=477), 349 (73.2%) reported mild to moderate symptoms (oFASS-3 grades 1/2) and 128 (26.8%) severe symptoms (oFASS-3 grade

3). **Table 2** shows the results of the univariable analyses for factors associated with severe symptoms. Patients with severe peach allergy were significantly more likely to have a higher age of onset of peach allergy and probable mugwort pollen and/or latex allergy. Patients with a severe peach allergy had significantly higher median IgE levels against both GRP allergens, Pru p 7 and Cup s 7.

In **Figure 4**, the results of the multivariable analyses are presented. In model 1 'age of onset', 'symptoms upon skin contact', 'mugwort pollen allergy', '*Parietaria* pollen allergy' and 'latex allergy', were selected as being positively associated with severity. In model 2, IgE against Japanese cedar pollen was added as a predictor to model 1 and in model 3 these were IgE levels to Pru p 4 and Pru p 7, while, 'symptoms upon skin contact' did not have added predictive value anymore and was excluded. Model 4 selected IgE against Pru p 7 (positively associated with severity) and IgE against Pru p 3 and 4 (inversely associated).

The area under the curve (AUC) of model 1 was 0.61 and of model 2 0.63. In model 3, the AUC increased to 0.73. The optimal sensitivity and specificity of the models were for model 1: 0.39 and 0.81, for model 2: 0.53 and 0.67 and for model 3: 0.63 and 0.75. Also model 4 had a poor predictive performance with an AUC of 0.63 (**Figure 4**) and a sensitivity and specificity of

	Model 1 Demographics + clinical history + pollen allergy	**Model 2 Model 1 + IgE to peach/cypress/ Japanese cedar extract**	**Model 3 Model 2 + IgE to peach components**	**Model 4 All peach components**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Age onset (y)	1.01 (0.94 - 1.09)	1.01 (0.93 - 1.10)	1.02 (0.94 - 1.12)	
Symptoms upon skin contact	1.35 (0.76 - 2.41)	1.34 (0.71 - 2.53)		
Mugwort pollen allergy	1.44 (0.85 - 2.44)	1.48 (0.83 - 2.63)	1.56 (0.77 - 3.15)	
Parietaria pollen allergy	1.64 (0.79 - 3.39)	1.71 (0.80 - 3.67)	2.08 (0.83 - 5.21)	
Latex allergy	1.51 (0.92 - 2.47)	1.56 (0.82 - 2.99)	2.01 (1.22 - 3.30)	
Japanese cedar		0.88 (0.51 - 1.53)	0.76 (0.46 - 1.26)	
Pru p 4			0.86 (0.62 - 1.21)	0.79 (0.59 - 1.06)
Pru p 7			1.79 (1.18 - 2.70)	1.65 (1.14 - 2.38)
Pru p 3				0.83 (0.68 - 1.02)
AUC	0.61 (0.61 - 0.61)	0.63 (0.62- 0.64)	0.73 (0.73 - 0.74)	0.63 (0.63 - 0.63)
AUC DBPCFC	0.50 (0.47 - 0.54)	0.61 (0.58 - 0.65)	0.80 (0.79 - 0.81)	0.65 (0.64 - 0.65)

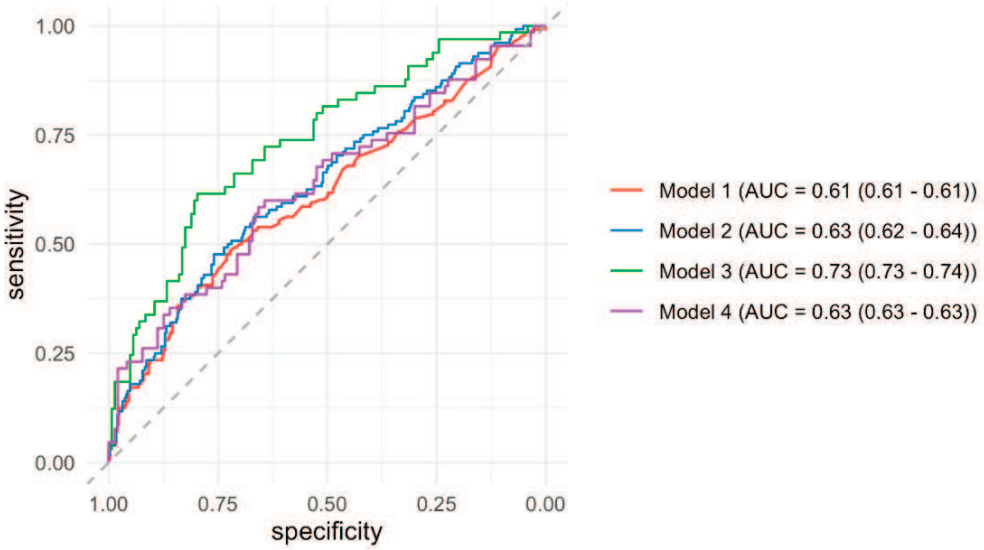


Figure 4. Prediction models and ROC-curves of the four models for predicting the severity of peach allergy. The area under the curve (95% confidence interval) is presented in the plot legends. For the DBPCFC subgroup only European patients were included. *Abbreviations: OR = odds ratio, CI = confidence interval*

respectively 0.58 and 0.66. When the model was built using European data only, very similar results were obtained with comparable AUC's (**Supplemental S7**).

The developed model was then applied to a subset of patients of the EuroPrevall study that had a positive DBPCFC (N=28) or had a history of severe anaphylaxis to peach (N=10). The AUCs of prediction model 1 was somewhat lower (AUC 0.50) and of model 3 higher (AUC 0.80). The AUCs of model 2 and 4 remained the same (**Figure 4**).

DISCUSSION

This study is the largest international study on peach allergy to date. Observed were regionally distinct sensitization patterns against peach, and correlations between peach and other allergens across Europe and Japan. A severity prediction model showed an AUC of 0.73 for distinguishing between mild-to-moderate and severe peach allergy.

Distribution of (co-)sensitization patterns and correlations

The different sensitization patterns to peach components among geographical areas have similarities with those reported for walnut, hazelnut and apple across Europe.[20–22] Sensitization to Pru p 1 (PR-10) dominated in birch pollen-exposed Northern and Central Europe and to Pru p 3 (LTP) in Southern Europe, but the latter was also common in North-

ern and Central Europe.[23] Almost 35% of the patients from Japan were (also) sensitized to Pru p 1. Since birch pollen are uncommon in Japan, this is likely due to sensitization to other tree pollen.[24] Sensitization to Pru p 3 in Japan was low, which might be explained by dietary differences.[25] Sensitization to Pru p 4 was variable but less clearly divided along a North-South axis[26], while Pru p 2 (TLP) sensitization seems to plays a negligible role in peach allergy, similar to what was reported for apple allergy.[22] Finally, Pru p 7 was barely recognized in the European centers, where exposure to cypress pollen for most centers, with the exception of Madrid and Milan[27], is generally low. For the Japanese patients, Pru p 7 was the dominant peach allergen, most likely driven by Japanese cedar pollen.[13]

Overall, IgE against Pru p 1 associated strongest with IgE to birch pollen and hazelnut and not so much to peach. Possible reason is that the hazelnut ImmunoCAP is spiked with rCor a 1. IgE against Pru p 3 showed the strongest (though moderate) association with IgE to walnut, followed by corn and lentil, but surprisingly not to peach and apple. Likely, the dominance of birch pollen and PR-10 sensitization has masked this association. When focusing on individual centers, IgE to Pru p 3 was strongly associated with that to peach and apple in Athens and Madrid, as reported by others.[22] Furthermore, these observations are suggestive for a more diverse spectrum of foods associated to LTP and peach sensitization.[28] Asero et al. also reported that IgE levels to peach in peach-allergic adults mono-sensitized to LTP strongly correlated with sensitization to walnut, hazelnut, lentil and corn.[29]

The GRPs Cry j 7 and Cup s 7 in pollen of Japanese cedar and common cypress, respectively, both members of the *Cupressaceae* family, are considered the primary sensitizers of Pru p 7.[12,13] We observed high correlations between IgE to Pru p 7 and Japanese cedar and cypress pollen. All Japanese peach allergic patients enrolled in the study were sensitized to Japanese cedar pollen, illustrating the dominant role of this pollen in peach allergy in Japan. Some caution is necessary, because Japanese peach allergic patients with oral symptoms only were excluded, which may have biased towards Pru p 7 sensitization (**Supplemental S7**). In the European centers, only 5.4% of the patients were sensitized to Pru p 7, which can be explained by low representation of centers with high exposure to cypress pollen.

Prediction of severity of peach allergy

A model combining age of onset of peach allergy, having probable mugwort pollen, *Parietaria* pollen and latex allergy together with IgE levels to Pru p 7, Japanese cedar pollen and to Pru p 4 was found to have the highest accuracy for predicting severity of peach allergy in patients with a probable peach allergy. Although it would be preferable to study this in patients with challenge-confirmed peach allergy, this group was too small. Therefore, as second best, the developed model was applied to the subset of patients

who had a positive DBPCFC or had severe anaphylaxis resulting in an even higher AUC, possibly supporting the generalizability of the model in peach allergic patients.

The explanation for IgE to mugwort and *Parietaria* being associated with severity is not really clear but has been reported earlier. Lyons et al. showed that mugwort allergy was the strongest predictor for severity of walnut allergy.[20] It may be that LTPs in weeds contribute to the LTP syndrome by diversifying epitope recognition, but we have not analyzed that at a molecular level. The predictive factor latex allergy has been reported in earlier studies on severity of hazelnut and peanut allergy[30,31] and has been suggested as part of a cluster of skin-related risk factors, also including atopic dermatitis and symptoms upon skin contact.[32] The latter was selected in models 1 and 2 in the present study.

In our study, Pru p 7 was the only peach allergen positively associated with severe peach allergy. The hypothesis that this was only due to the biased Japanese selection could be rejected, because a post-hoc analysis showed that this effect was also seen in Northern and Southern Europe (**Supplemental S7**). Pru p 7 is a GRP with high stability to heat and resistance to digestive enzymes. Such properties might be related to the potential to induce severe allergic reactions, as described a.o. for LTP.[33,34] Other studies in Southern France[13] and Japan[35] also found a relationship between Pru p 7 and severe peach allergy, although not absolute, as was demonstrated in a study from Italy.[36] It would be interesting to study the effect of Pru p 7 in more regions.

In the model, both IgE to Japanese cedar and IgE to Pru p 4 were inversely related to severe peach allergy which is in line with earlier reports.[11] However, the inverse association of IgE to Japanese cedar pollen with severity may seem unexpected, being a primary source of sensitization to Pru p 7. Because exposure to Japanese cedar pollen is virtually absent in Europe, primary sensitization to cypress pollen is likely the basis of the observed (cross-)sensitization to Japanese cedar. Nevertheless, in most cities exposure to cypress pollen is low. Therefore, sensitization to major allergens like Cup s 1 will likely dominate, and significant sensitization to Cup s 7 will only occur in areas with high exposure, as described for olive (Ole e 7 and 9)[37] and mugwort pollen (Art v 3)[38].

Another surprising observation was that IgE to Pru p 3 tended to be inversely associated with severity. This may be explained by the regional differences; a univariable logistic regression with regional interaction term showed a trend towards a positive association between the level of IgE to Pru p 3 and severity in Southern Europe while in Northern Europe this tended to be inversely associated (1.05 *vs.* 0.86, p = 0.135)(**Supplemental S7**). The lack of significance can be attributed to the low number of patients in Northern Europe with severe symptoms (n = 27). It is unclear why sensitization to Pru p 3 in one area is associated with severity whereas in another area it is inversely associated.

Due to selection the data in this study could not be generalized to the entire population. However, the aim of this study was to identify serologic, demographic, and clinical risk factors associated with severity of peach allergy to improve diagnosis, and not to provide solid unbiased prevalence data.

Conclusion

To conclude, we showed that sensitization to Pru p 7 was low and variable in the European centers but dominant in Japan and correlations between IgE to peach components with pollen and foods are similar to those of other plant source foods. A model with reasonable predictive accuracy showed that a higher age of onset of peach allergy, having probable mugwort pollen, *Parietaria* pollen and latex allergy together with sensitization to Japanese cedar, Pru p 4 and Pru p 7 were the best predictors of a severe peach allergy.

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SUPPLEMENTAL

Supplemental S1. Selection procedure of European patients

A potential weakness of the current study is that we were forced to select patients for CRD due to limited availability of component ImmunoCAPs. The aim of the study was to identify serologic, demographic, and clinical risk factors associated with severity of peach allergy to improve diagnosis, and not to provide solid unbiased prevalence data. With this aim in mind, we purposely chose for a biased selection. Our first decision was to include available sera of all patients that had undergone a DBPCFC and all that had severe anaphylaxis (N=51). Then, our idea was to try to increase the chance to include patients with likely sensitivity to allergens of which we expected numbers to be low, i.e., Pru p 2 and Pru p 7. From earlier EuroPrevall microarray analyses that included the apple TLP Mal d 2, we knew that positivity was scarce. Therefore, we selected all available sera with proven IgE reactivity to Mal d 2 (N=10). To increase the chance of "catching" Pru p 7 positive patients, we took the following approach. We selected 139 sera with significant IgE titers against cypress pollen but low to profilin and CCD (based on the EuroPrevall microarray results). The rationale behind this was that this would remove most sera positive for cypress pollen (mainly) based on profilin and/or CCD cross-reactivity, thereby increasing the chance of finding primary sensitization to cypress allergens, in particular to Cup s 7. To make sure that other components would not end up being under-represented, we then selected a group (N=102) with the following CRD profiles observed in the earlier EuroPrevall microarray analyses: typical PR-10 sera, profilin sera, mixed PR-10/profilin reactors, mixed PR-10/LTP reactors, LTP sera and sera with clear IgE to peach but no clear recognition of components on the microarray. Finally, we evaluated the country representation in this obtained selection and added sera from relatively under-represented centers with higher IgE reactivities to peach (N=164; see also **Table 1**). With that we had a selection of 466 sera, together with the 34 sera from Japan, bringing it to a total of 500. Based on the inclusion criteria of this study, 26 patients with CRD were excluded.

Selection procedure for CRD analyses is appropriate for purpose of the study

Clearly, the selection process has not been random. Including all challenged/anaphylactic patients was done to have the opportunity to perform a sub-analysis of patients with truly confirmed peach allergy, and to increase the number of patients with oFASS-3 grade 3. As a result of that, there was a bias towards these more severe phenotypes (10/38 with severe anaphylaxis) in the sub-analysis with the patients who had a positive DBPCFC or had a history of severe anaphylaxis. This has probably provided us sufficient power to perform a meaningful evaluation of the prediction models developed on the basis of probable food allergy.

A potential risk of our approach to increase the chance of picking up Pru p 2 and Pru p 7

sensitization was that patients with sensitization to Pru p 1, Pru p 3, Pru p 4 and/or CCD would be under-represented. To find out whether this was the case, we compared the selected and the unselected sera with respect to their IgE responses to peach, to cypress pollen (ImmunoCAP) and to components on EuroPrevall microarray, i.e., to CCD, to profilin (Mal d 4), to Pru p 1 and to Pru p 3. In figure S1.1, the percentage of sera positive to these allergens are compared between the selected and unselected groups. As expected, there is a bias towards selection of positive cypress pollen sera. The selection further resulted in a slightly lower frequency of Pru p 1 and profilin positives. For Pru p 3, the selection process actually resulted in a significantly higher number of positives.

Next, we compared the strength of the positive IgE responses between the selected and unselected patients. As can be seen in **Figures S1.2-1.4**, in all cases the IgE responses in the selected sera were higher than in the unselected. Taken together, the targeted selection has not resulted in under-representation of sensitization to other allergens than Pru p 2 and Pru p 7.

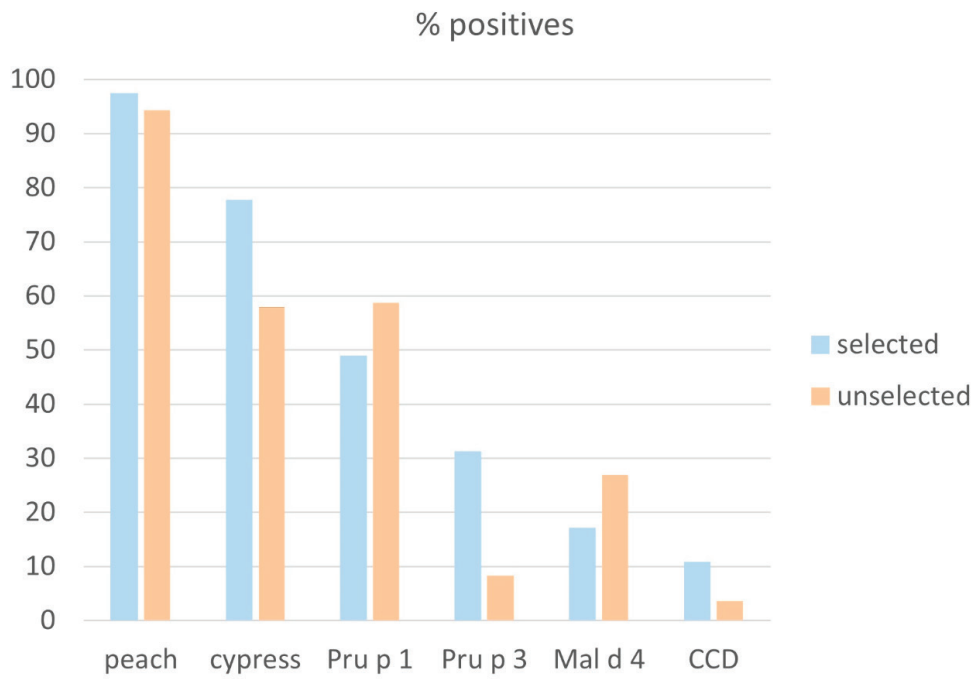


Figure S1.1. Percentages with a positive IgE response (≥ 0.1 kU/L) in selected versus unselected patients

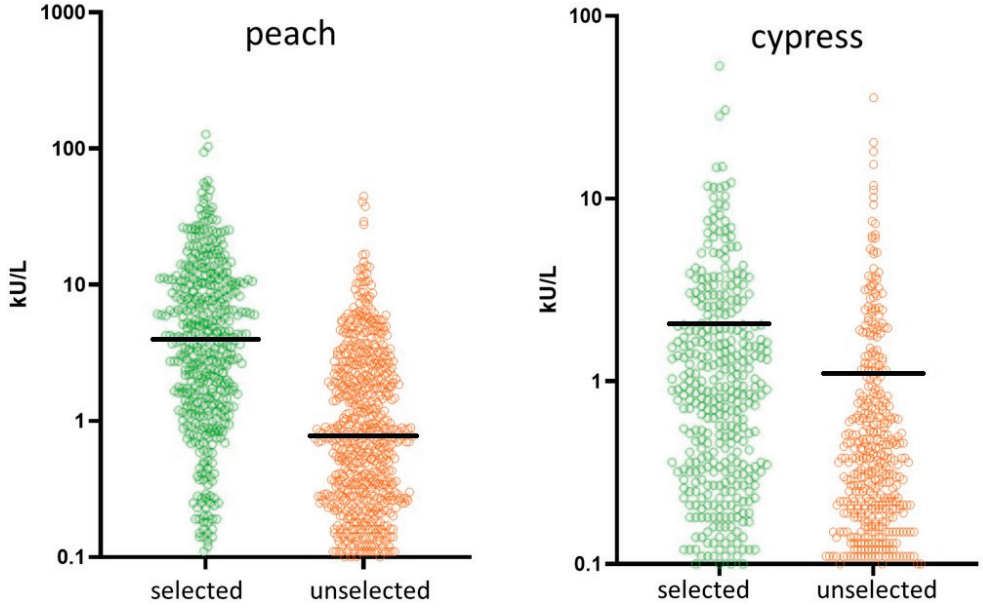


Figure S1.2. IgE against peach and cypress pollen in selected and unselected patients

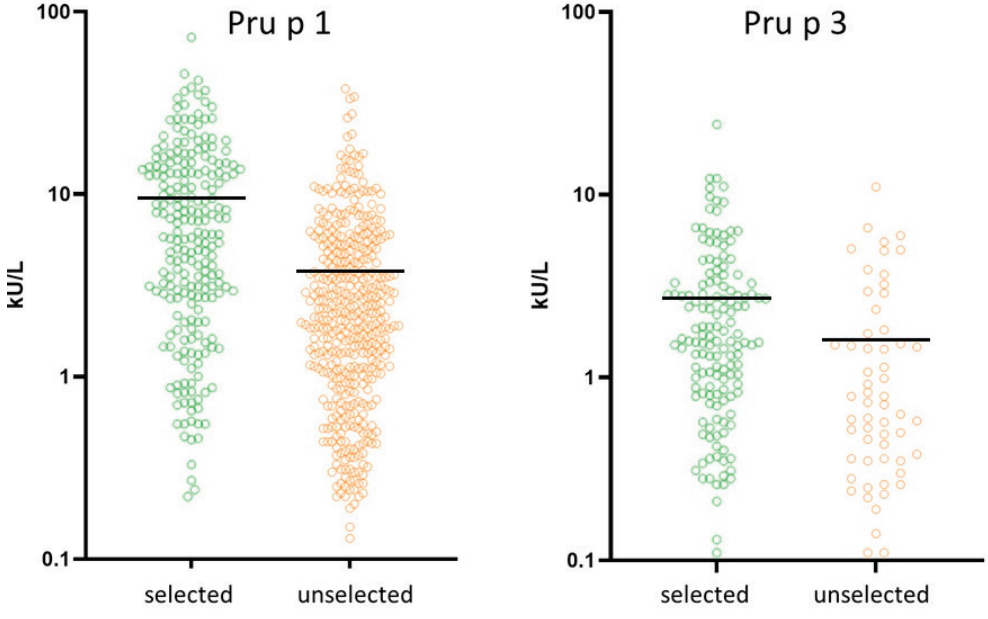


Figure S1.3. IgE against Pru p 1 and Pru p 3 in selected versus unselected patients

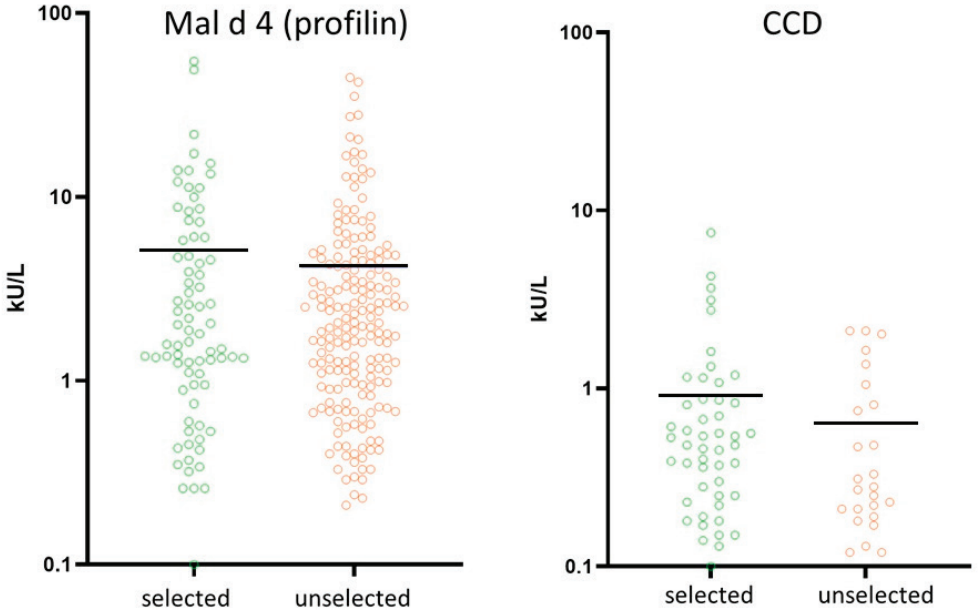


Figure S1.4. IgE against profilin and CCD in selected versus unselected patients

We further checked whether there were significant differences in patient characteristics between those selected for CRD and those not selected (**Table S1**). There were some significant differences that mostly were in line with having biased the selection for CRD towards patients with probable peach allergy (34.9% versus 44.9%) and with a more severe phenotype (grade 2/3: 20.7% versus 29.6%): younger age, age of onset < 18 and a positive skin test and ImmunoCAP for peach. The aim of our studies being diagnostic in nature, an increased power for more severe phenotypes was considered an advantage. On the other hand, the biased selection makes conclusions of more epidemiological nature (prevalence of outcomes) less reliable.

Table S1. Characteristics of patients in which components have (not) been measured

	No CRD (N=757)	**CRD (N=474)**	**P-value**
Demographics			
Age visit (years) Mean (SD)	29.5 (± 15.3)	27.2 (± 14.3)	**0.007**
Age onset (years) Mean (SD)	22.0 (± 14.4)	19.7 (± 13.2)	0.061
Age onset <18	142/341 (41.6%)	112/214 (52.3%)	**0.018**
Female sex	451/757 (59.6%)	274/495 (55.4%)	0.133
Symptoms			
Severity oFASS-3			**<0.001**
No symptoms of peach	414 (53.7%)	256 (54.0%)	
1	185 (24.4%)	78 (16.5%)	
2	80 (10.6%)	71 (15.0%)	
3	78 (10.1%)	69 (14.6%)	

Table S1. Characteristics of patients in which components have (not) been measured (*continued*)

	No CRD (N=757)	CRD (N=474)	P-value
Probable peach allergy	264 (34.9%)	213 (44.9%)	**0.001**
Sensitisation to			
Peach SPT extract	113/741 (14.9%)	156/429 (36.4%)	**<0.001**
Peach peel SPT fresh food	35/84 (41.7%)	28/45 (62.2%)	**0.041**
Peach pulp SPT fresh food	51/100 (51.0%)	32/52 (61.5%)	0.286
Positive to peach extract	528/649 (81.4%)	429 /481 (89.2%)	**<0.001**

Supplemental S2. Missing data

In Table S2 an overview is given of the missing data in the data collection of the 477 patients with probable peach allergy. A maximum of 44 data points (9.4%) for a single variable was missing from the data set. For these missing data points, multiple imputation was performed for building multivariable prediction models (See Section S3.)

Table S2. Missing data

	Missing (N = 477) N(%)
Age of inclusion (y)	1 (0.2%)
Age of onset (y)	7 (1.5%)
Sex	0 (0%)
Family atopy	40 (8.5%)
Symptoms upon skin contact	44 (9.4%)
Birch allergy	21 (4.5%)
Grass allergy	21 (4.5%)
Mugwort allergy	16 (3.4%)
Parietaria allergy	18 (3.8%)
Planetree allergy	18 (3.8%)
Cypress allergy	22 (4.7%)
Olive allergy	22 (4.7%)
Ragweed allergy	18 (3.8%)
Chenopodium allergy	18 (3.8%)
Dust allergy	61 (13%)
Latex allergy	7 (1.5%)
SPT peach extract	42 (8.9%)
IgE level peach extract	6 (1.3%)

Supplemental S3. Detailed information about constructing the models

Multiple imputation was performed (20 imputations by chained equations using the R package 'mice'). IgE levels to the different peach components were log-transformed to approximate a normal distribution of these variables. Based on earlier studies on walnut[20]

and hazelnut[30], a Least Absolute Shrinkage and Selection Operator (Lasso) regression was performed for all 20 imputed datasets (using R package 'glmnet'). A ten-fold cross-validation (CV) was used to determine the optimal penalization. With the optimal LASSO penalization, a LASSO model was fitted on the full imputed datasets. This resulted in 20 prediction models, that did not all contain the same predictors. Predictor variables selected in at least 14 of the 20 imputed datasets were then included as predictor variables in a logistic regression with Ridge penalization (optimal penalization was determined using ten-fold CV) analysis per imputed dataset. Resulting regression coefficients and standard errors were then pooled using Rubin's rule to obtain final estimates for Odds Ratio's with 95% confidence intervals (CI).

Supplemental S4. Distribution of sensitized and symptomatic patients

Supplemental S4. Distribution of sensitized and symptomatic patients

	Sensitisation	No sensitisation	Total
Reported symptoms	477	84	561
No reported symptoms	670	0	670
Total	1147	84	1231

Supplemental 5. Sensitization patterns

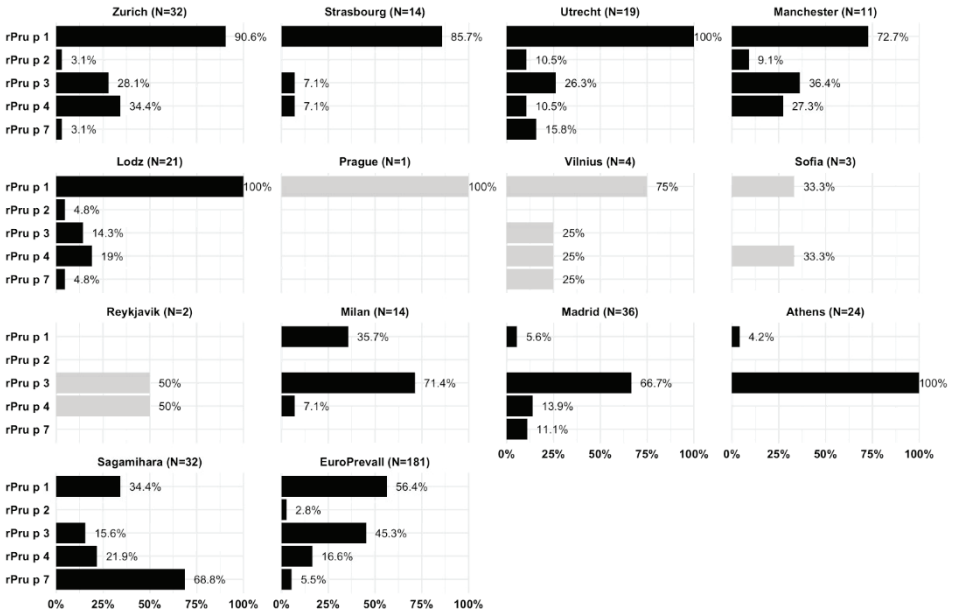


Figure S5.1. IgE to peach amongst patients with probable peach allergy across Europe and in Japan
In this figure only IgE to positive peach components (IgE levels ≥ 0.35 kU_A/L) is shown. Centers with numbers of patients N < 10 are shown in grey.

Table S5.2. Number of patients with mono- or co- sensitization

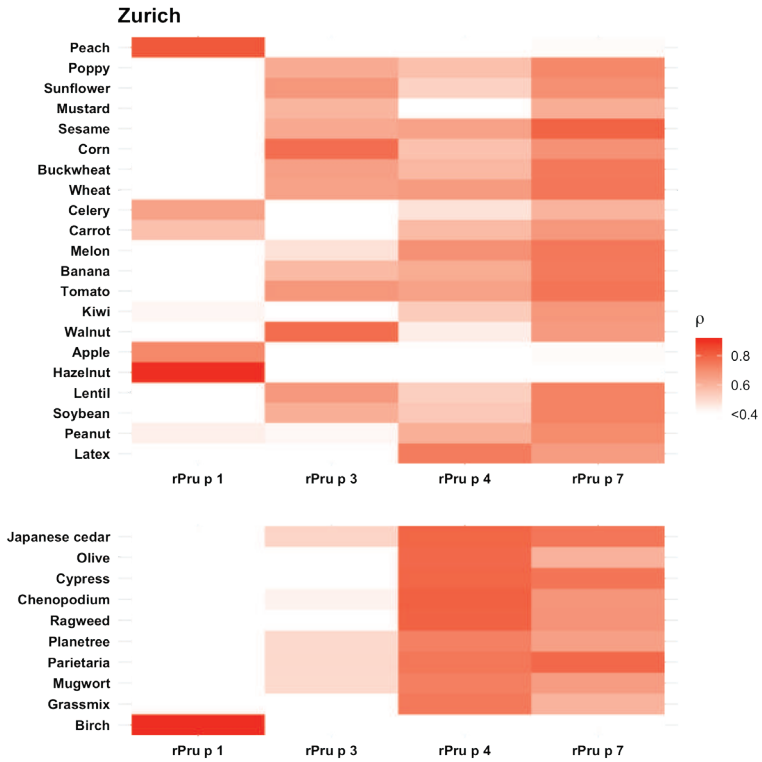
					Overall	Probable peach allergy		
						oFASS-3 grade 1	oFASS-3 grade 2	oFASS-3 grade 3
rPru p 1	**rPru p 2**	**rPru p 3**	**rPru p 4**	**rPru p 7**	**N=474**	**N = 77**	**N = 69**	**N = 67**
x					143	28	22	21
x	x				3	0	0	0
x		x			40	3	4	4
x			x		32	8	1	3
x				x	7	1	1	4
x	x	x			0	0	0	0
x	x		x		1	0	0	0
x	x			x	0	0	0	0
x		x	x		13	2	3	1
x		x		x	3	0	0	0
x			x	x	1	0	0	1
x	x	x	x		2	1	0	1
x		x	x	x	1	1	0	0
x	x		x	x	0	0	0	0
x	x	x		x	1	0	0	1
x	x	x	x	x	7	1	1	0
	x				1	0	0	0
	x	x			2	0	0	0
	x		x		0	0	0	0
	x			x	0	0	0	0
	x	x	x		1	0	0	0
	x	x		x	0	0	0	0
	x		x	x	0	0	0	0
	x	x	x	x	2	0	0	0
		x			119	23	23	14
		x	x		10	1	1	0
		x		x	5	1	1	0
		x	x	x	2	0	0	0
			x		20	4	3	2
			x	x	2	1	1	1
				x	18	0	6	11
				None	38	3	2	3

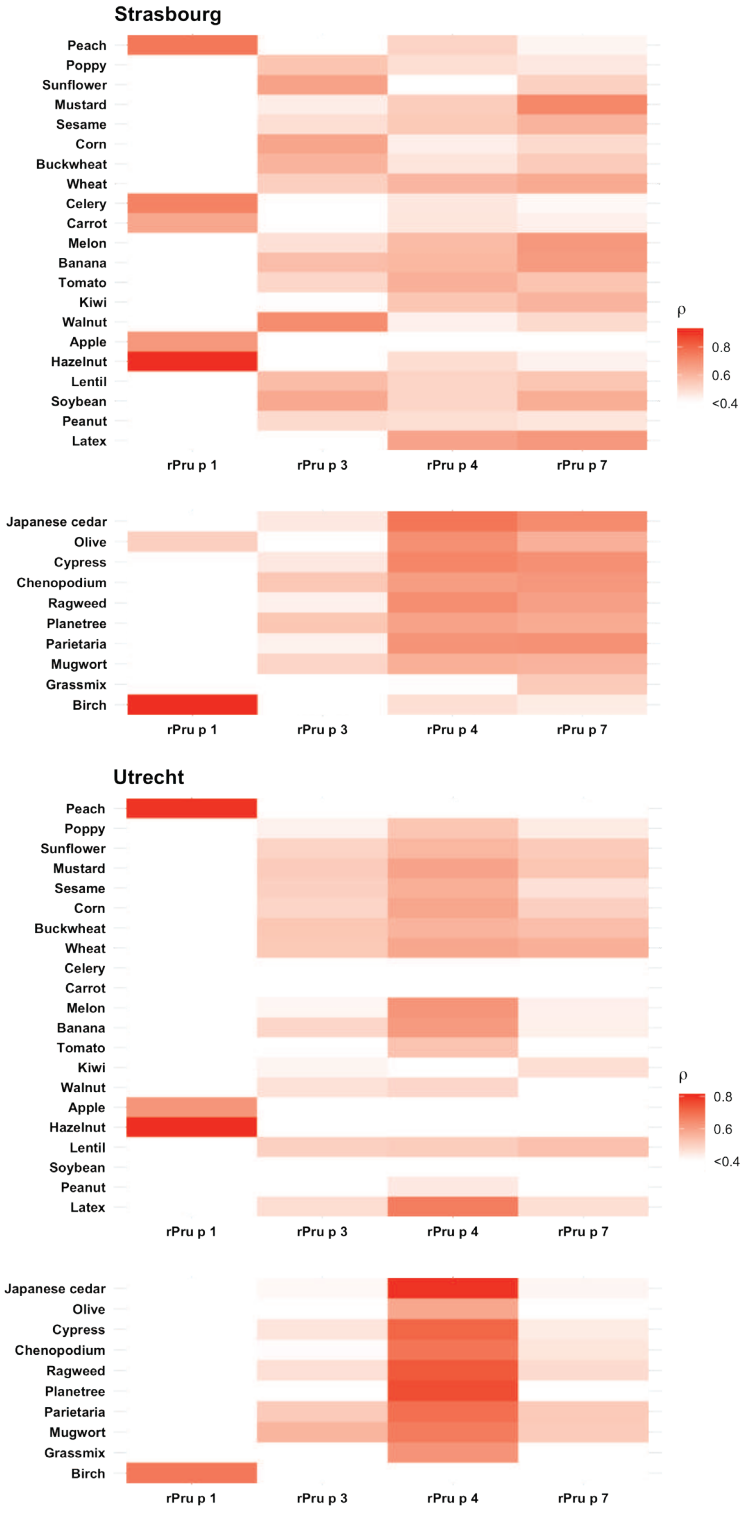
X indicates having a sensitization to the peach component (IgE levels ≥ 0.35 kU_A/L)

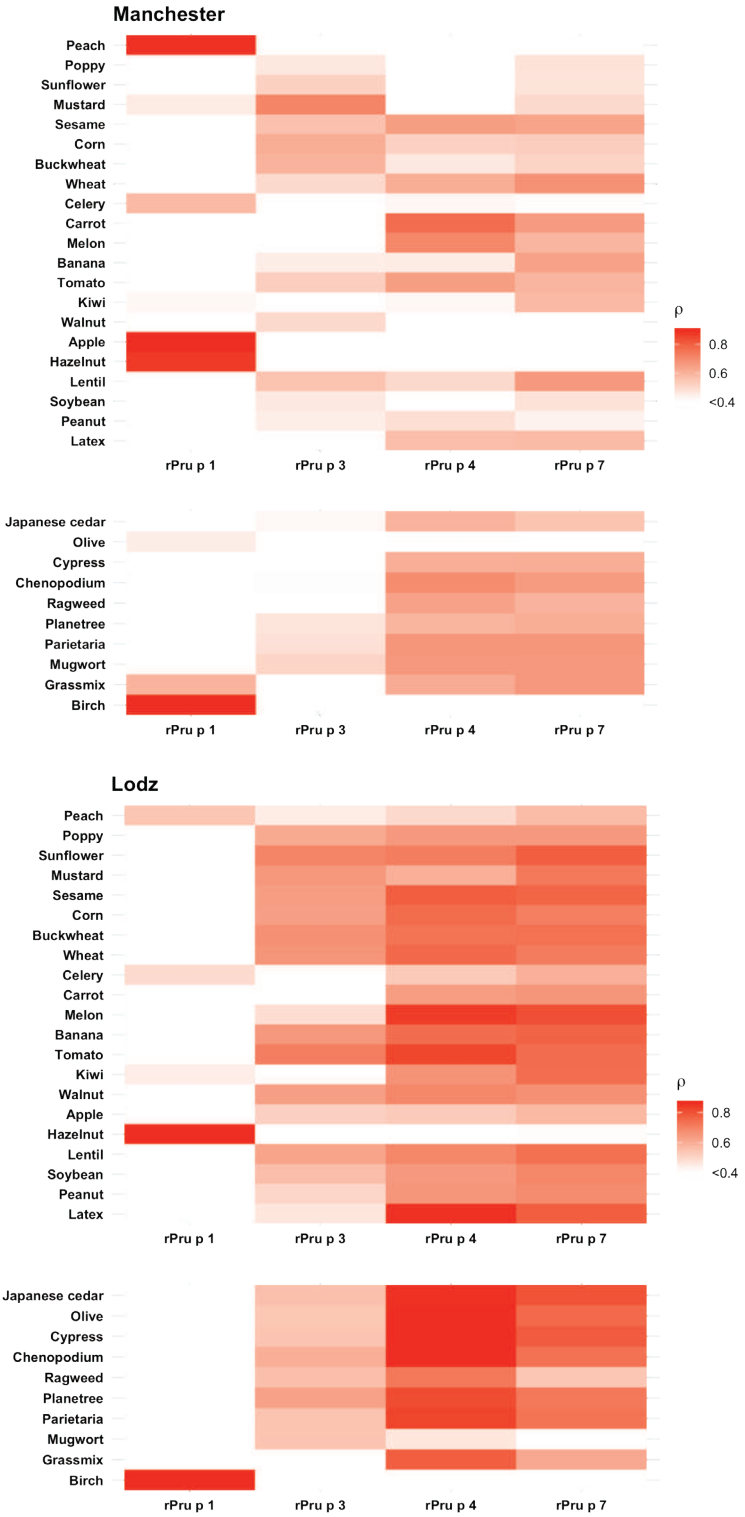
Table S5.3. Number of patients with mono-sensitization per center

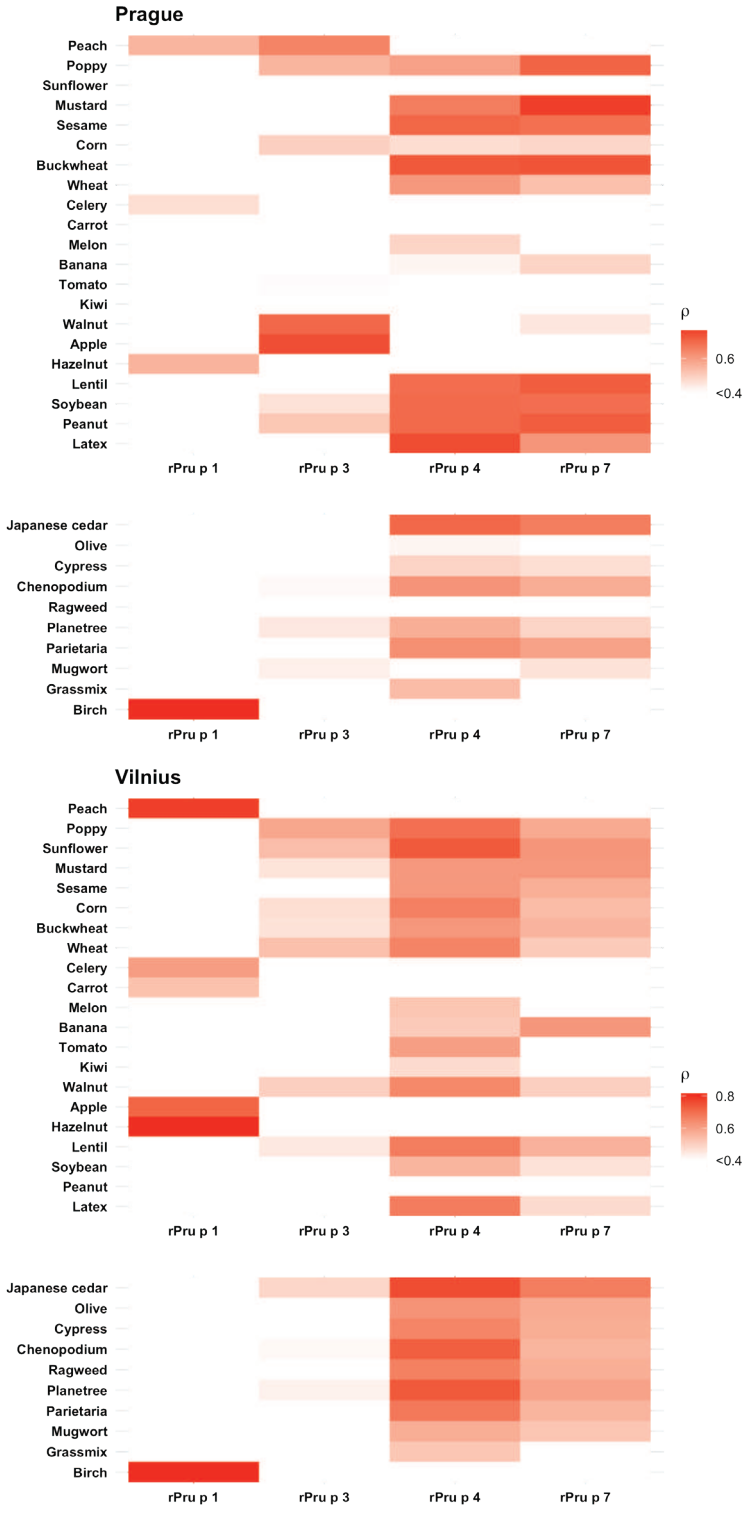
	Pru p 1	Pru p 2	Pru p 3	Pru p 4	Pru p 7
Overall (N=474)	143	1	119	20	18
Utrecht (N=39)	18	0	1	1	1
Zurich (N=61)	24	0	4	0	0
Madrid (N=60)	6	0	29	6	2
Athens (N=71)	1	0	55	1	0
Lodz (N=50)	26	0	5	1	0
Manchester (N=22)	9	0	2	1	0
Sofia (N=13)	1	1	1	1	0
Praque (N=20)	6	0	2	0	0
Vinius (N=32)	18	0	2	0	0
Milan (N=20)	4	0	11	1	0
Strasbourg (N=32)	21	0	3	0	0
Reykjavik (N=20)	8	0	3	6	0
Sagamihara (N=34)	1	0	1	2	15

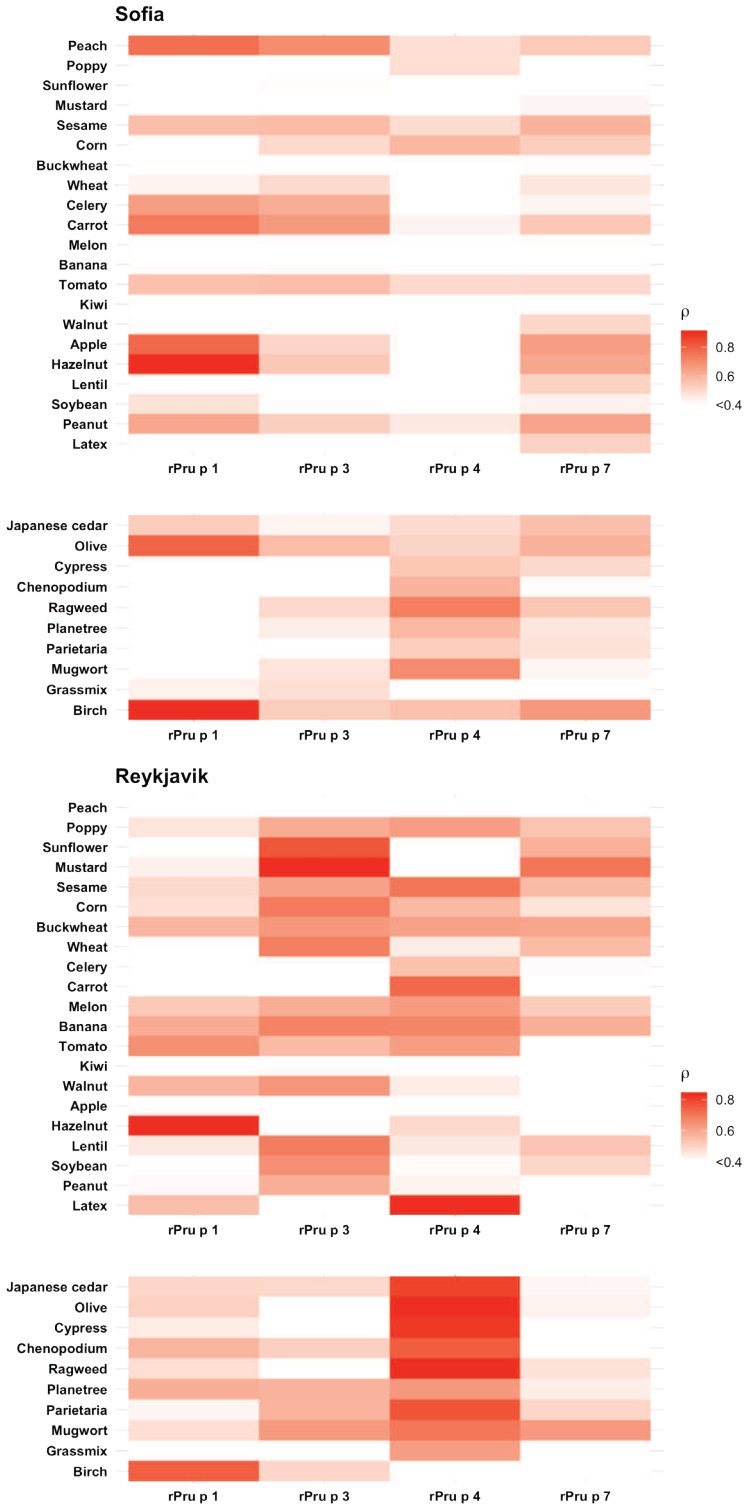
Supplemental 6. Correlations between IgE to components and to food and pollen

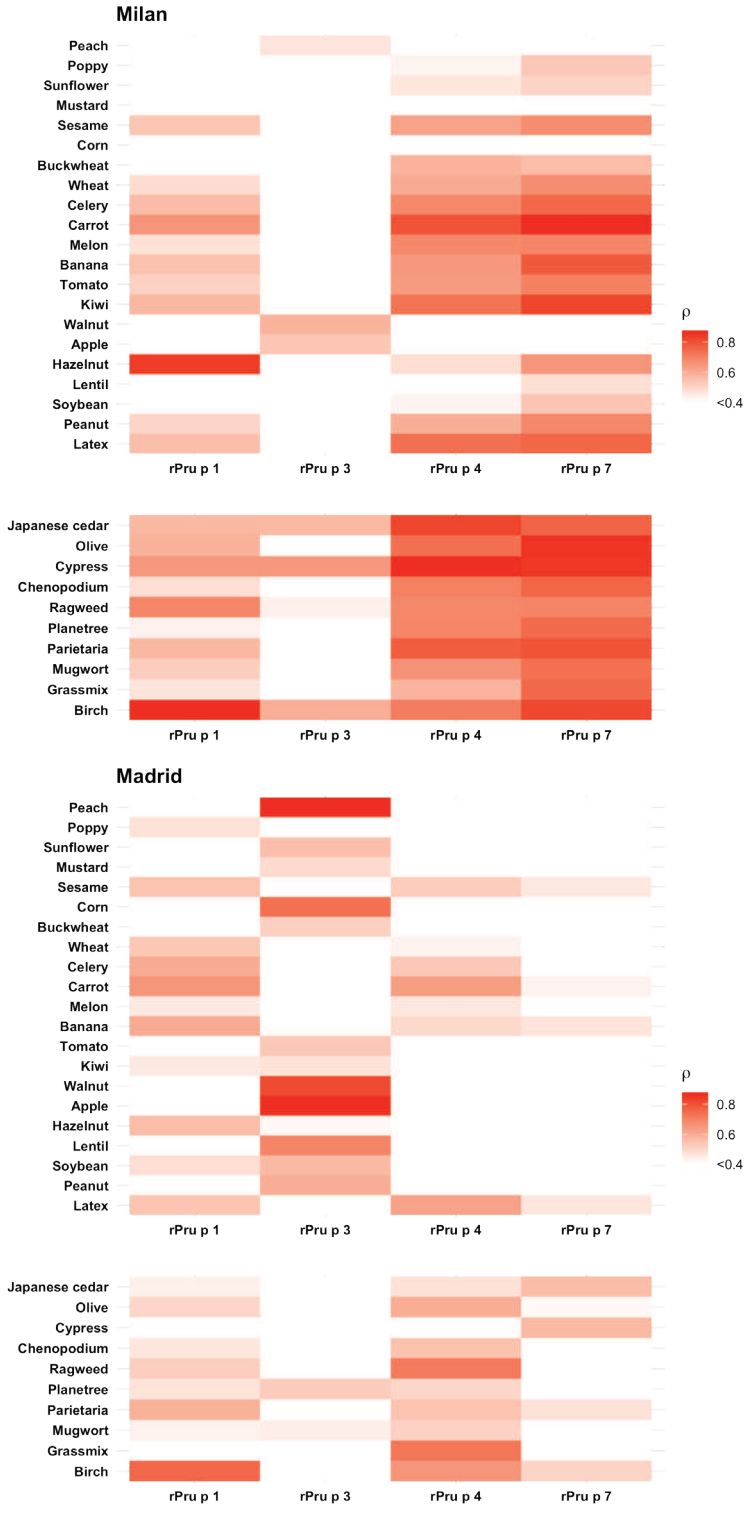



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Corn
Buckwheat
Wheat
Celery
Carrot
Melon
Banana
Tomato
Kiwi
Walnut
Apple
Hazelnut
Lentil
Soybean
Peanut
Latex
rPru p 1
rPru p 3
rPru p 4
rPru p 7
ρ
0.8
0.6
<0.4
Japanese cedar
Olive
Cypress
Chenopodium
Ragweed
Planetree
Parietaria
Mugwort
Grassmix
Birch
rPru p 1
rPru p 3
rPru p 4
rPru p 7

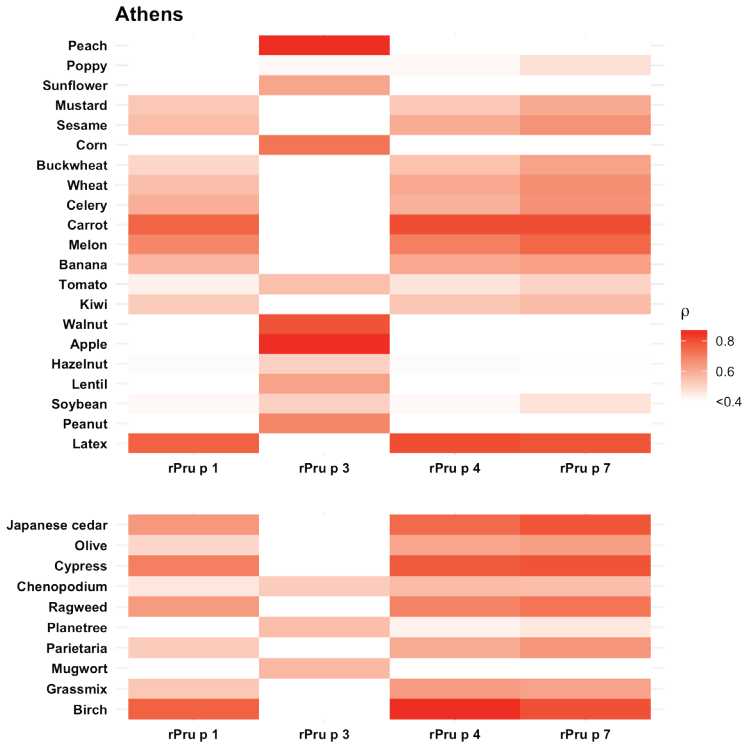


Figure S6.1. Correlations between IgE levels to peach components and to pollen and plant foods for individual centers with at least 10 patients completing CRD testing
Abbreviations: CRD = component resolved diagnostics

Supplemental 7. Univariable regression and Lasso models based on European patients only

To investigate whether the identified risk factors for severity were biased by inclusion of the Japanese center, we performed the univariable analyses for three European selections: all patients (Table S7.1), for Southern European patients from Athens, Milano and Madrid (Table S7.2) and for Northern European patients from the other nine centers (Table S7.3)

Overall, the associations with risk of severe reactions were very similar to the ones observed for the analyses performed for the whole population (Europe plus Japan). Risk factors identified, with variable degree of significance due to decreased power, were age/age of onset, symptoms upon skin contact, latex allergy and mugwort allergy. Interestingly, in the sub-analysis for Southern Europe the inverse association of sensitization to Pru p 3 (and to Japanese cedar pollen) turned into a positive association, although this did not reach significance. This of course fits with the well accepted concept of Pru p 3 sensitization being a Mediterranean risk factor for severe reactions to peach.

Table S7.1. Predictors for severity of peach allergy in *European* patients with probable peach allergy

	oFASS-3 grades 1 or 2 (N=335)	oFASS-3 grade 3 (N=110)	P	Univariable OR (95% CI)
Demographics				
Age of inclusion (y) mean (±SD)	30.5 (±13.5)	33.5 (±13.3)	**0.040**	**1.02 (1.00 – 1.03)**
Female sex	225 (67.2%)	73 (66.4%)	0.877	0.97 (0.61 – 1.53)
Clinical history				
Age of onset (y)	20.0 (±12.8)	22.7 (±12.8)	0.054	1.02 (1.00 – 1.03)
Family atopy	202 (64.7%)	63 (62.4%)	0.666	0.90 (0.57 – 1.45)
Symptoms upon skin contact	53 (17.0%)	25 (24.8%)	0.087	1.60 (0.92 – 2.73)
Pollen allergies				
Birch	216 (66.3%)	75 (69.4%)	0.542	1.16 (0.73 – 1.87)
Grass	193 (59.4%)	73 (67.0%)	0.160	1.39 (0.88 – 2.21)
Mugwort	30 (9.1%)	20 (18.3%)	**0.010**	**2.23 (1.20 – 4.10)**
Parietaria	12 (3.7%)	9 (8.4%)	0.054	2.41 (0.96 – 5.87)
Plane tree	10 (3.1%)	6 (5.6%)	0.231	1.89 (0.63 – 5.20)
Cypress	10 (3.1%)	6 (5.6%)	0.225	1.90 (0.63 – 5.25)
Olive	35 (10.8%)	10 (9.5%)	0.717	0.87 (0.40 – 1.77)
Ragweed	24 (7.3%)	9 (8.3%)	0.723	1.16 (0.49 – 2.49)
Chenopodium	26 (8.0%)	12 (11.2%)	0.302	1.46 (0.69 – 2.95)
Other				
House dust mite allergy	79 (25.2%)	27 (26.2%)	0.844	1.05 (0.63 – 1.73)
Latex allergy	15 (4.6%)	12 (11.0%)	**0.019**	**2.59 (1.15 – 5.71)**
Cat/dog sensitization	106 (38.4%)	40 (42.6%)	0.478	1.19 (0.74 – 1.91)
Japanese cedar sensitization	55 (42.0%)	16 (32.7%)	0.256	0.67 (0.33 – 1.32)
Peach sensitization, median (Q1 – Q3)*				
SPT peach extract	0.00 (0.00 - 0.68)	0.00 (0.00 – 0.58)	0.940	1.01 (0.71 – 1.41)
IgE level peach extract	2.41 (0.93 – 5.40)	2.43 (1.26 – 5.32)	0.702	1.08 (0.73 – 1.60)
IgE level rPru p 1	2.72 (0.01 – 20.97)	8.10 (0.01 – 23.62)	0.454	1.09 (0.88 – 1.35)
IgE level rPru p 2	0.03 (0.01 – 0.06)	0.02 (0.02 – 0.07)	0.651	1.17 (0.58 – 2.31)
IgE level rPru p 3	0.24 (0.06 – 3.80)	0.11 (0.04 – 2.54)	0.376	0.86 (0.62 – 1.19)
IgE level rPru p 4	0.03 (0.01 – 0.12)	0.03 (0.02 – 0.10)	0.593	0.90 (0.60 – 1.30)
IgE level rPru p 7	0.04 (0.03 – 0.08)	0.04 (0.03 – 0.11)	0.275	1.41 (0.74 – 2.63)
Cupressaceae sensitization, median (Q1 – Q3)*				
IgE level cypress pollen extract	0.19 (0.05 – 0.91)	0.19 (0.07 – 1.25)	0.601	0.93 (0.70 – 1.23)
IgE level rCup s 7	0.02 (0.01 – 0.04)	0.02 (0.01 – 0.06)	0.824	0.95 (0.56 – 1.52)
IgE level Japanese cedar	0.29 (0.06 – 1.48)	0.17 (0.04 – 0.70)	0.271	0.81 (0.56 – 1.17)

Table S7.2. Predictors for severity of peach allergy in *Southern European* patients with probable peach allergy

	oFASS-3 grades 1 or 2 (N=123)	oFASS-3 grade 3 (N=27)	P	Univariable OR (95% CI)
Demographics				
Age of inclusion (y) mean (±SD)	29.2 (±13.9)	31.8 (±12.4)	0.377	1.01 (0.98 – 1.05)
Female sex	78 (63.4%)	15 (55.6%)	0.447	0.72 (0.31 – 1.70)
Clinical history				
Age of onset (y)	18.1 (±13.5)	18.5 (±14.7)	0.891	1.00 (0.97 – 1.03)
Family atopy	66 (56.9%)	14 (53.9%)	0.777	0.88 (0.38 – 2.10)
Symptoms upon skin contact	39 (34.2%)	9 (37.5%)	0.759	1.15 (0.45 – 2.84)
Pollen allergies				
Birch	36 (29.3%)	5 (18.5%)	0.261	0.55 (0.17 – 1.46)
Grass	70 (56.9%)	16 (59.3%)	0.823	1.10 (0.48 – 2.63)
Mugwort	3 (2.4%)	1 (3.7%)	0.714	1.54 (0.07 – 1.26)
Parietaria	6 (4.9%)	3 (11.1%)	0.230	2.44 (0.49 – 9.94)
Plane tree	2 (1.6%)	1 (3.7%)	0.498	2.33 (0.11 – 2.52)
Cypress	6 (4.9%)	3 (11.1%)	0.230	2.44 (0.49 – 9.94)
Olive	32 (26.0%)	8 (30.8%)	0.620	1.26 (0.48 – 3.11)
Ragweed	15 (12.2%)	3 (11.1%)	0.875	0.90 (0.20 – 3.00)
Chenopodium	1 (0.8%)	1 (3.7%)	0.280	4.69 (0.18 – 12.13)
Other				
House dust mite allergy	17 (13.8%)	0 (0%)	-	-
Latex allergy	4 (3.3%)	3 (11.1%)	0.099	3.72 (0.70 – 1.79)
Cat/dog sensitization	26 (23.9%)	3 (11.1%)	0.264	0.48 (0.11 – 1.54)
Japanese cedar sensitization	25 (42.4%)	7 (46.7%)	0.765	1.19 (0.37 – 3.74)
Peach sensitization, median (Q1 – Q3)*				
SPT peach extract	0.81 (0.00 – 1.28)	1.00 (0.00 – 1.50)	0.096	1.51 (0.93 – 2.46)
IgE level peach extract	2.01 (0.83 – 5.38)	1.75 (0.97 – 6.63)	0.678	1.16 (0.58 – 2.38)
IgE level rPru p 1	0.01 (0.00 – 0.02)	0.00 (0.00 – 0.01)	0.203	0.54 (0.16 – 1.15)
IgE level rPru p 2	0.03 (0.01 – 0.04)	0.02 (0.02 – 0.03)	0.748	1.30 (0.26 – 6.57)
IgE level rPru p 3	3.05 (0.45 – 9.73)	6.41 (1.46 – 20.85)	0.316	1.38 (0.76 – 2.76)
IgE level rPru p 4	0.02 (0.01 – 0.06)	0.02 (0.01 – 0.03)	0.366	0.63 (0.19 – 1.51)
IgE level rPru p 7	0.03 (0.03 – 0.05)	0.03 (0.03 – 0.08)	0.190	1.79 (0.71 – 4.39)
Cupressaceae sensitization, median (Q1 – Q3)*				
IgE level cypress pollen extract	0.31 (0.06 – 1.38)	0.27 (0.07 – 0.85)	0.590	0.87 (0.53 – 1.43)
IgE level rCup s 7	0.02 (0.01 – 0.03)	0.02 (0.01 – 0.05)	0.278	1.42 (0.72 – 2.66)
IgE level Japanese cedar	0.36 (0.02 – 1.38)	0.23 (0.08 – 1.35)	0.575	1.20 (0.65 – 2.33)

Table S7.3. Predictors for severity of peach allergy in *Northern European* patients with probable peach allergy

	oFASS-3 grades 1 or 2 (N=212)	oFASS-3 grade 3 (N=83)	P	Univariable OR (95% CI)
Demographics				
Age of inclusion (y) mean (±SD)	31.2 (±13.2)	34.1 (±13.5)	0.095	1.02 (1.00 – 1.04)
Female sex	147 (69.3%)	58 (69.9%)	0.928	1.03 (0.56 – 1.80)
Clinical history				
Age of onset (y)	21.1 (±12.2)	24.1 (±11.9)	0.061	1.02 (1.00 – 1.04)
Family atopy	136 (69.4%)	49 (65.3%)	0.521	0.83 (0.48 – 1.47)
Symptoms upon skin contact	14 (7.1%)	16 (20.8%)	**0.002**	**3.43 (1.58 – 7.52)**
Pollen allergies				
Birch	180 (88.7%)	70 (86.4%)	0.598	0.81 (0.38 – 1.82)
Grass	123 (60.9%)	57 (69.5%)	0.173	1.46 (0.85 – 2.56)
Mugwort	27 (13.2%)	19 (23.2%)	**0.039**	**1.99 (1.02 – 3.81)**
Parietaria	6 (2.9%)	6 (7.5%)	0.097	2.68 (0.81 – 8.81)
Plane tree	8 (3.9%)	5 (6.3%)	0.402	1.62 (0.48 – 5.05)
Cypress	4 (1.9%)	3 (3.8%)	0.379	1.98 (0.38 – 9.17)
Olive	3 (1.5%)	2 (2.5%)	0.555	1.72 (0.22 – 10.59)
Ragweed	9 (4.3%)	6 (7.4%)	0.303	1.75 (0.57 – 5.03)
Chenopodium	25 (12.3%)	11 (13.8%)	0.733	1.14 (0.52 – 2.39)
Other				
House dust mite allergy	62 (32.6%)	27 (35.5%)	0.651	1.14 (0.65 – 1.98)
Latex allergy	11 (5.3%)	9 (11.0%)	0.096	2.19 (0.85 – 5.50)
Cat/dog sensitization	80 (47.9%)	37 (52.1%)	0.553	1.18 (0.68 – 2.07)
Japanese cedar sensitization	30 (41.7%)	9 (26.5%)	0.133	0.50 (0.20 – 1.20)
Peach sensitization, median (Q1 – Q3)*				
SPT peach extract	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.803	1.10 (0.51 – 2.20)
IgE level peach extract	2.48 (1.11 – 5.46)	2.76 (1.34 – 5.23)	0.999	1.00 (0.62 – 1.63)
IgE level rPru p 1	16.3 (5.12 – 42.54)	20.30 (7.65 – 33.2)	0.900	0.97 (0.63 – 1.57)
IgE level rPru p 2	0.03 (0.02 – 0.07)	0.03 (0.02 – 0.08)	0.958	0.98 (0.44 – 2.12)
IgE level rPru p 3	0.09 (0.04 – 0.31)	0.06 (0.03 – 0.27)	0.409	0.79 (0.44 – 1.36)
IgE level rPru p 4	0.05 (0.02 – 0.26)	0.03 (0.02 – 0.14)	0.539	0.87 (0.55- 1.33)
IgE level rPru p 7	0.05 (0.03 – 0.09)	0.04 (0.03 – 0.12)	0.922	1.05 (0.41 – 2.55)
Cupressaceae sensitization, median (Q1 – Q3)*				
IgE level cypress pollen extract	0.14 (0.05 – 0.65)	0.15 (0.07 – 0.62)	0.929	1.02 (0.71 – 1.45)
IgE level rCup s 7	0.02 (0.01 – 0.04)	0.02 (0.01 – 0.06)	0.241	0.65 (0,30 – 1.31)
IgE level Japanese cedar	0.28 (0.07 – 1.58)	0.15 (0.04 – 0.50)	0.083	0.65 (0.39 – 1.05)

To further take away the concern that the exclusion of Japanese patients with mild oral allergy had biased the analyses towards an important role of Pru p 7, we performed the univariable regression on European and Japanese patients after excluding all European patients with oFASS-3 grade 1 (Table S7.4). This made both selections comparable. Although borderline significant, also in this sub-analysis IgE against Pru p 7 was identified as risk factor (p=0.097) whereas IgE against Pru p 3 tended to be inversely associated (p=0.078).

Table S7.4. Predictors for severity of peach allergy of European patients with probable peach allergy *without oFASS-3 grade 1*

	oFASS-3 grade 2 (N=120)	**oFASS-3 grade 3 (N=128)**	**P**	**Univariable OR (95% CI)**
Demographics				
Age of inclusion (y) mean (±SD)	26.2 (15.2)	32.2 (14.7)	**0.002**	**1.03 (1.01 – 1.05)**
Female sex	74 (61.7%)	85 (66.4%)	0.437	1.23 (0.73 – 2.07)
Clinical history				
Age of onset (y)	16.6 (14.0)	22.7 (13.9)	**0.001**	**1.03 (1.01 – 1.05)**
Family atopy	66 (60.1%)	74 (64.3%)	0.557	1.18 (0.68 – 2.02)
Symptoms upon skin contact	22 (21.0%)	25 (22.7%)	0.753	1.11 (0.58 – 2.13)
Pollen allergies				
Birch	55 (48.7%)	75 (62.5%)	**0.034**	**1.76 (1.05 – 2.97)**
Grass	55 (48.7%)	73 (60.8%)	0.063	1.64 (0.98 – 2.77)
Mugwort	11 (9.6%)	20 (16.4%)	0.129	1.84 (0.85 – 4.15)
Parietaria	1 (0.9%)	9 (7.4%)	**0.038**	**9.08 (1.67 – 168.8)**
Plane tree	2 (1.8%)	6 (5.0%)	0.195	2.92 (0.66 – 20.23)
Cypress	1 (0.9%)	6 (5.0%)	0.093	6.22 (1.04 – 118.5)
Olive	9 (8.0%)	10 (8.4%)	0.903	1.06 (0.41 – 2.77)
Ragweed	3 (2.6%)	9 (7.6%)	0.103	3.03 (0.88 – 13.91)
Chenopodium	9 (8.0%)	12 (9.9%)	0.588	1.28 (0.52 – 2.37)
Other				
House dust mite allergy	22 (22.2%)	27 (26.2%)	0.509	1.24 (0.65 – 2.39)
Latex allergy	7 (6.0%)	12 (9.4%)	0.317	1.64 (0.64 – 4.59)
Cat/dog sensitization	35 (42.2%)	40 (42.6%)	0.959	1.02 (0.56 – 1.85)
Japanese cedar sensitization	15 (27.8%)	16 (32.7%)	0.590	1.26 (0.54 – 2.95)
Peach sensitization, median (Q1 – Q3)*				
SPT peach extract	0.00 (0.00 – 1.04)	0.00 (0.00 – 0.58)	0.054	0.67 (0.45 – 1.00)
IgE level peach extract	2.54 (1.06 – 5.67)	2.03 (0.91 – 5.19)	0.939	0.98 (0.65 – 1.48)
IgE level rPru p 1	0.09 (0.01 – 16.29)	1.68 (0.01 – 22.21)	0.506	1.08 (0.86 – 1.36)
IgE level rPru p 2	0.03 (0.01 – 0.07)	0.02 (0.01 – 0.07)	0.925	0.97 (0.50 – 1.86)
IgE level rPru p 3	0.31 (0.07 – 3.08)	0.07 (0.04 – 1.41)	0.078	0.73 (0.51 – 1.03)

Table S7.4. Predictors for severity of peach allergy of European patients with probable peach allergy *without oFASS-3 grade 1* (*continued*)

	oFASS-3 grade 2 (N=120)	oFASS-3 grade 3 (N=128)	P	Univariable OR (95% CI)
IgE level rPru p 4	0.04 (0.01 – 0.13)	0.03 (0.02 – 0.08)	0.550	0.89 (0.61 – 1.30)
IgE level rPru p 7	0.04 (0.03 – 0.12)	0.06 (0.03 – 0.47)	0.097	1.39 (0.95 – 2.07)
Cupressaceae sensitization, median (Q1 – Q3)*				
IgE level cypress pollen extract	0.15 (0.05 – 0.81)	0.19 (0.07 – 0.62)	0.618	1.03 (0.72 – 1.48)
IgE level rCup s 7	0.02 (0.01 – 0.08)	0.03 (0.01 – 0.15)	0.227	1.19 (0.90 – 1.59)
IgE level Japanese cedar	0.11 (0.02 – 0.74)	0.17 (0.04 – 0.70)	0.935	1.02 (0.65 – 1.60)

A further analysis was performed to evaluate whether there was a difference in effect of IgE levels to the 5 peach components and severity in Southern (centers in Madrid, Milan and Athens) versus Northern Europe (centers in Lodz, Manchester, Prague, Reykjavik, Sofia, Strasbourg, Utrecht, Vilnius and Zürich) versus Japan, by a univariate logistic regression with a regional interaction term (Table S7.5). Results showed that in Southern Europe a trend towards a positive association between the level of IgE to Pru p 3 and severity whereas in Northern Europe this trend was inversely associated with severity (OR=0.79 *vs* OR=1.38, respectively). This difference was not significant, but the total number of patients in Northern Europe with severe symptoms (oFASS-3 grade 3) was only 27, so power is likely insufficient. In all three regions, IgE levels to Pru p 7 tended to be positively associated with severe peach allergy, again likely due to insufficient power not reaching significance. For Cup s 7 an even stronger association with severity was found in Southern Europe and Japan while in Northern Europe this was inversely associated. Also these associations did not reach significance.

Table S7.5. Effect of peach components in Northern and Southern Europe and Japan

	Reference								
	Northern Europe			**Southern Europe**			**Japan**		
	Log(OR)(SE)	OR	p	Log(OR)(SE)	OR	p	Log(OR)(SE)	OR	p
IgE level Pru p 1	-0.03 (0.23)	0.97	0.90	-0.58 (0.53)	0.54	0.27	-0.35 (0.41)	0.69	0.39
IgE level Pru p 2	-0.02 (0.40)	0.98	0.96	0.28 (0.91)	1.30	0.76	-1.02 (0.91)	0.35	0.76
IgE level Pru p 3	-0.24 (0.29)	0.79	0.41	0.56 (0.43)	1.38	0.19	-0.24 (0.80)	0.62	0.76
IgE level Pru p 4	-0.14 (0.22)	0.87	0.54	-0.33 (0.57)	0.63	0.56	-0.55 (0.46)	0.50	0.23
IgE level Pru p 7	0.05 (0.47)	1.05	0.92	0.31 (0.59)	1.42	0.60	0.54 (0.64)	1.79	0.40
IgE level Cup s 7	-0.44 (0.37)	0.64	0.24	0.78 (0.49)	1.41	0.11	0.72 (0.47)	1.40	0.12

Finally, to check whether models based on data from European patients would give similar results, Lasso regression analysis was performed on all patients excluding the

Japanese patients (Table S7.6). Selected outcomes and resulting AUCs were very similar to the ones found for the whole population including the Japanese patients.

Table S7.6. Prediction models for severity of peach allergy in European centers only

	Model 1 Demographics + clinical history + pollen allergy	**Model 2** Model 1 + IgE to peach/cypress/ Japanese cedar extract	**Model 3** Model 2 + IgE to peach components	**Model 4** All peach components
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Age onset (y)	1.01 (0.9 – 1.08)	1.01 (0.93– 1.01)	1.02 (0.92– 1.14)	
Mugwort pollen allergy	1.38 (0.90– 2.21)	1.44 (0.84– 2.47)	1.44 (0.68– 3.03)	
Parietaria pollen allergy	1.58 (0.86– 2.92)	1.70 (0.80– 3.62)	2.01 (0.80– 3.45)	
Latex allergy	1.54 (0.95– 2.52)	1.64 (0.81– 3.30)	2.06 (1.23– 3.46)	
Japanese cedar		0.88 (0.50– 1.56)	0.75 (0.43– 1.30)	
Pru p 4			0.87 (0.63– 1.20)	0.80 (0.58– 1.10)
Pru p 7			1.80 (1.05– 3.09)	1.62 (1.09– 2.43)
Pru p 3				0.84 (0.67– 1.05)
AUC	0.60 (0.59– 0.60)	0.62 (0.61– 0.63)	0.73 (0.73- 0.74)	0.63 (0.63– 0.63)

4

Severity of almond allergy and diagnostic value of almond extract in adults

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Introduction

No studies investigated the clinical characteristics and diagnostics of almond allergy in a well-defined adult population.

Objectives

This study aims to 1) describe the clinical characteristics of adults with suspected almond allergy, in particular its severity, 2) describe the clinical characteristics of another group of patients who underwent an open food challenge (OFC) for almond and 3) evaluate the diagnostic value of almond-specific IgE (sIgE) and SPT for the presence of almond allergy.

Methods

Adult patients reporting symptoms to almond within two hours after ingestion (n=221) and who underwent an OFC with raw almond (n=90) were included. The diagnostic value of almond-sIgE, SPT, and their combination was assessed using receiver operating characteristics (ROC) analysis.

Results

Of the 221 patients, 29.1% reported severe symptoms. Of the 90 OFCs, 33.3% were positive, of which 36.7% were severe. All patients with a positive OFC reported subjective symptoms and 56.7% reported also objective symptoms. Co-allergy to hazelnut, apple, and walnut were most common as was cosensitisation to hazelnut extract and PR-10 components. Co-allergy and cosensitisation to other nuts were less common. ROC-analysis showed low area under the curves (AUCs) in predicting almond allergy for almond sIgE (0.54; 95% CI=0.38 – 0.71), SPT to almond extract (0.64; 95% CI=0.49– 0.80), and their combination (0.68; 95% CI=0.53 - 0.83).

Conclusion

Almond allergy is severe in one third of the patients. Both almond-sIgE and SPT have a low diagnostic value for predicting almond allergy. Therefore, there is a high need for better diagnostic tools such as improved extracts and almond components-based tests.

INTRODUCTION

Almond is worldwide the most consumed nut (botanically related to the *Rosaceae* family[1]), with nearly 1.6 million metric tons of almond consumption per year.[2] A recent systematic review showed that only one study investigated the point prevalence of a probable almond allergy (reported symptoms with matching sensitization), which was 0.8% in children. The overall point prevalence of self-reported almond allergy was 2.0% in children and adults.[3]

In order to give well-founded advice to adult patients about their almond allergy, knowledge about the clinical characteristics of almond allergy is necessary (e.g. severity, co-allergy and cosensitisation for other foods). Most of the studies on almond allergy are performed in children and less in known about almond allergy in adults. Furthermore, the reports on the severity of almond allergy are contradictory: two studies stated that almond causes mostly mild symptoms[4,5], but more severe reactions, including anaphylaxis, can also occur.[6,7] In addition, little information is known about co-allergies and co-sensitization in almond allergic patients.

Of the seven studies that performed oral food challenges (OFC) with almond, six showed strikingly low positive OFC results with 0%-5.0% of the patients being positive [5–10], and one a remarkably high proportion, with 52.4%[4] of the patients being positive. Most of these OFCs were performed in children. Furthermore, the OFC material consisted mainly of processed almond (e.g. almond butter or flour) or of an unknown processed form. In Europe, around 60% of the almonds are consumed as snacks which can be eaten raw.[11] As thermal processing can influence the allergenicity, it is important to know the reaction to raw almonds.[12] As far as we know, there are no studies that show OFC results with raw almonds in a large adult population with a suspected almond allergy.

Although OFCs are the gold standard for allergy diagnosis, they are time-consuming, costly, and labor-intensive with risks of severe reactions.[13] Specific IgE (sIgE) testing (e.g., ImmunoCAP) and skin prick testing (SPT), both with almond extract, are less invasive and can easily be performed. A few studies investigated the diagnostic accuracy of these tests in children, showing poor accuracy and limited usefulness.[13] However, one study showed an AUC of 0.87 for sIgE to almond extract, but then the estimated 95% predicted probability of positive OFC would have a cut-off value of 167 kU_A/L which is much higher than any of the observed almond IgE results. No studies have described the diagnostic accuracy of almond extract in an adult population.

This highlights the scarcity and contradiction of data on the clinical characteristics and the diagnostic value of sIgE and SPT with almond extract in an adult population with (suspected) almond allergy. Therefore, this study aims to 1) describe the clinical charac-

teristics of adults with suspected almond allergy, 2) describe the clinical characteristics of adults who underwent an OFC with almond and 3) evaluate the diagnostic value of sIgE and SPT to almond extract in patients who underwent an OFC to almond.

METHODS

Subject selection

All consecutive adult patients who visited the Department of Dermatology and Allergy of the University Medical Centre Utrecht (UMCU), The Netherlands, were included if they reported symptoms to almond within two hours after ingestion. Another group of adult patients who underwent OFCs to almond were included to evaluate the diagnostic value of almond-sIgE and SPT. Data was collected between August 2012 and April 2024. This study was approved by the local ethical committee (protocol number 23-233).

Data collection

Clinical questionnaire

Since 2018, physicians at the allergology outpatient clinic of UMCU have systematically collected clinical data during the first outpatient clinic visit using a standardized questionnaire. Data was collected regarding age, sex, allergic comorbidities (allergic rhinoconjunctivitis [AR] for birch- and grass pollen, allergic asthma [AA], and atopic dermatitis [AD]), reported symptoms, severity of reaction(s), time between ingestion and first onset of symptoms to almond, and reported concomitant symptoms to Brazil nut, cashew nut, hazelnut, macadamia nut, pecan nut, pistachio, walnut, peanut, soy, apple, banana, kiwi, melon, peach, carrot, celery, tomato, and pine nut. These foods have been selected on the basis of phylogenetic and birch pollen similarity.

Diagnostic tests

Sensitization was measured by skin prick tests (SPT) and/or sIgE tests. SPTs (ALK, Madrid, Spain) were performed with almond extract, birch- and grass pollen, tree nut extracts (Brazil nut, cashew nut, hazelnut, macadamia nut, pecan nut, pistachio, and walnut), legume extracts (peanut and soy), fruit extracts (apple, banana, kiwi, melon, peach, and tomato), vegetable extracts (carrot and celery) and pine nut extract. sIgE tests (ImmunoCAP platform, Thermo Fisher Scientific, Uppsala, Sweden) were performed to the same allergens mentioned above, to melon extract, and to the components Bet v 1, Ana o 3, Cor a 1/9/14, Pru p 3, Ara h 2/6/8 and Gly m 4.

A wheal diameter ≥ 3 mm and sIgE levels ≥ 0.35 kU_a/L, were considered positive, respectively.

Open food challenge

OFCs followed a local protocol based on PRACTALL guidelines.[14] Three protocols were used during the study period. The first protocol consisted of 0.01 gram - 0.03 gram - 0.1 gram – 0.3 gram – 1 gram – 3 grams - 10 grams and 20 grams of almond. The second protocol consisted of 0.014 gram – 0.048 gram – 0.14 gram – 0.48 gram – 1.43 grams - 4.76 grams and 14.29 grams of almond, and the last one consisted of one dose of 5 gram almond. These steps were comparable for raw almond, blanched, and roasted almond. Every 20-30 minutes a new dose was administered. All patients were observed for at least 2 hours after administering the last portion or when a reaction was ceased.

An OFC was considered positive if subjective symptoms persisted for at least 45 minutes or if objective allergic reactions occurred. An expert panel decided on the OFC's outcome including the degree of severity.

Raw almond was defined as almond with peel, not having undergone any form of processing (e.g. pasteurizing, blaching, roasting) after harvesting. Processed almond was defined as almond that underwent one or multiple types of processing after harvesting.

Severity of symptoms

Allergic reactions were graded using the Ordinal Food Allergy Severity Score - 3 (oFASS-3) scoring, with oFASS-3 grade 1 being mild (isolated oropharyngeal symptoms), oFASS-3 grade 2 being moderate (symptoms of the skin, eye, upper airways, digestive system and/or uterus systems with or without grade 1 symptoms), and oFASS-3 grade 3 being severe (symptoms of the lower airways, cardiovascular and/or nervous system with or without grade 1 and/or 2 symptoms).[14]

Data analysis

Patient characteristics including type of reported symptoms to almond, severity of symproms, sIgE and SPT values to almond extract, reported allergic symptoms to other foods, and co-sensitization to other allergen sources were described.

Differences in patient characteristics between those who underwent an OFC with a positive and a negative result and between those who were objectively allergic and those with exclusively subjective symptoms were compared using a T-test, Mann-Whitney U tests, Chi-square tests or Fishers exact test. Differences in sIgE levels and SPT sizes between allergic patients with mild, moderate or severe symptoms were compared using one-way ANOVA. P-values ≤ 0.05 were considered statistically significant.

To investigate the diagnostic value of sIgE and SPT testing, logistic regression analyses were performed with 'positive OFC' respectively 'objective symptoms' as outcome and sIgE level, SPT wheal size and their combination as independent variables. Odds ratios

and the area under the curve (AUC) of the receiver operating characteristic (ROC) were reported as a measure of (diagnostic) accurarcy. The DeLong's test was used to statistically compare the AUCs for sIgE and SPT. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the clinical cutoff points and the optimal cutoff point defined by the Youden Index were also calculated.

Statistical analysis was performed using IBM SPSS statistics (version 29.0.1) and MedCalc[15].

RESULTS

Patients with reported symptoms to almond

Clinical characteristics

A total of 221 patients reported symptoms after almond consumption, of which 71.5% were female (**Table 1**). Of the atopic comorbidities, birch pollen allergy was the most common (80.2%). Around one third of the patients reported mild (35.0%), moderate (35.9%) or severe symptoms (29.1%) to almond and most patients (80.5%) reported at least oropharyngeal symptoms.

The correlation between almond-sIgE levels and wheal sizes was 0.64 ($p<0.001$). In total, only 40.4% of the patients were sensitized by sIgE to almond extract, and 71.8% of the patients were sensitized by SPT with almond extract.

Table 1. Characteristics of patients with suspected almond allergy

Total	**221**	**(100.0)**
Sex, Female	158	(71.5)
Age, mean (SD)	36.2	(13.4)
Atopic comorbidities		
Allergic asthma	104	(49.1)
Atopic dermatitis	126	(59.2)
Birch pollen allergy	134	(80.2)
Grass pollen allergy	93	(73.8)
Time of onset of symptoms		
< 5 min	172	(88.7)
5 - 30 min	17	(8.8)
30 - 120 min	5	(2.6)
Severity of symptoms		
Mild	72	(35.0)
Moderate	74	(35.9)
Severe	60	(29.1)

Table 1. Characteristics of patients with suspected almond allergy (*continued*)

Total	**221**	**(100.0)**
Level of sIgE/Wheal size of almond extract		
Level of sIgE, median (IQR)	0.24	(0.06 - 0.80)
Wheal size, mean (SD)	4.2	(3.2)
Sensitization to almond extract		
ImmunoCAP	69	(40.4)
SPT	126	(72.0)
Almond OFC result		
Allergic	5	(38.5)
Tolerant	8	(61.5)

All measurements are in N (%) or otherwise specified
Abbreviations: *N = Number; SD = Standard Deviation; IQR = Interquartile Range; sIgE = specific Immunoglobulin E; SPT = Skin Prick Test; OFC = Open Food Challenge*

Reported symptoms and co-sensitization to other foods

Most patients who reported allergic symptoms to almond also reported symptoms to other foods with hazelnut being the most commonly reported (69.9%), followed by apple (62.6%) and walnut (56.3%) (**Figure 1**). Symptoms to other tree nuts were less common (range 12.1%-28.6%).

Regarding severity, severe symptoms were most frequently reported for pecan nut (47.6%) and pine nut (42.3%) (and celery, but based on very low numbers), followed by tree nuts and peanut, which were reported in around 30% of the patients (**Figure 1)**.

Many patients who reported symptoms to almond also demonstrated sensitization to one or more other allergen extracts and components (**Table 2**). Regarding tree nuts, sensitization to hazelnut extract was the most common (86.5%), even more than to almond extract. Sensitisation to extracts of other tree nuts was less common (range 10.8%-35.4%). Regarding individual components, sensitization to PR-10 proteins (Ara h 8, Cor a 1, Bet v 1, and Gly m 4) were most common (range 81.0%-95.0%).

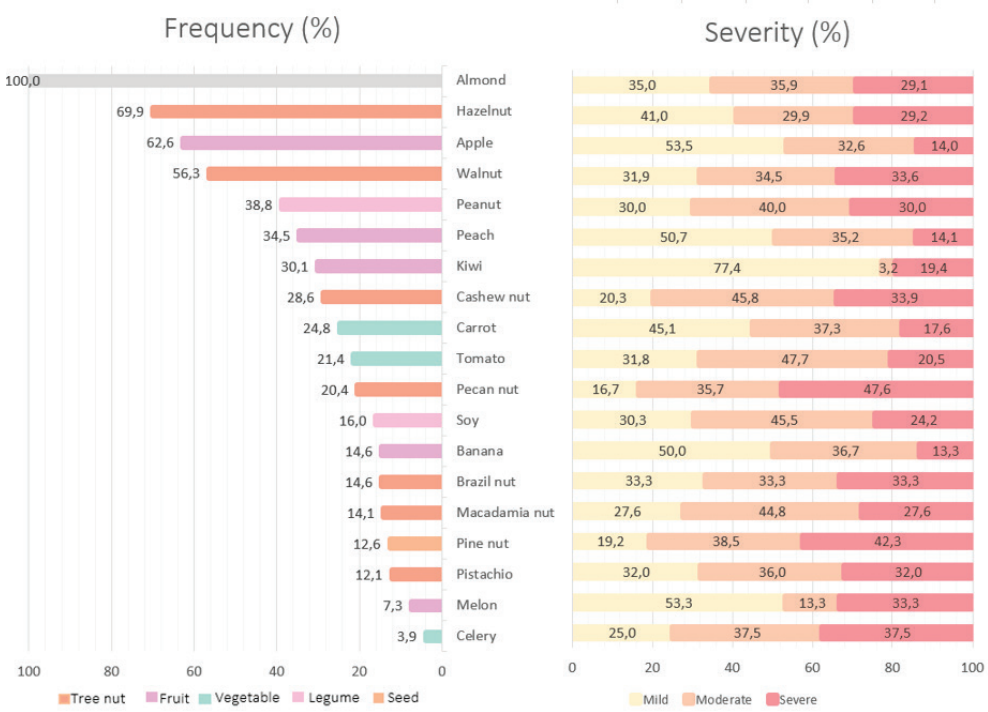


Figure 1. Frequency and concomitant severity of reported symptoms to other foods when symptoms were also reported to almonds

Table 2. Heatmap of percentage sensitizated to other allergens in patients with symptoms to almond

	sIgE	SPT	Combination*
Pollen			
Birch pollen	82.3	82.5	78.1
Bet v 1	87.5	-	87.5
Grass pollen	73.3	74.1	74.7
Tree nuts			
Almond	40.4	72.0	81.9
Brazil nut extract	10.6	10.0	10.8
Cashew nut extract	17.0	14.5	17.5
Ana o 3	14.3	-	14.3
Hazelnut extract	86.7	81.3	86.5
Cor a 1	85.9	-	85.9
Cor a 9	10.2	-	10.2
Cor a 14	11.0	-	11.0
Macadamia nut extract	22.5	25.0	22.4
Pecan nut extract	15.9	10.4	15.6
Pistachio extract	35.8	0.0	35.4

Table 2. Heatmap of percentage sensitizated to other allergens in patients with symptoms to almond (*continued*)

	sIgE	SPT	Combination*
Walnut extract	27.8	33.6	35.0
Fruits			
Apple extract	92.6	77.8	67.3
Banana extract	33.3	8.8	11.4
Kiwi extract	45.5	76.1	74.0
Melon extract	16.7	-	16.7
Peach extract	94.4	64.4	68.5
Pru p 3	8.0	-	8.0
Tomato extract	0.0	51.9	51.1
Vegetables			
Carrot extract	44.4	37.2	44.4
Celery extract	0.0	27.9	27.7
Legumes			
Peanut extract	68.4	61.3	65.4
Ara h 2	33.0	-	33.0
Ara h 6	30.0	-	30.0
Ara h 8	81.0	-	81.0
Soy extract	30.0	-	28.1
Gly m 4	95.0	-	95.0
Seeds			
Pine nut extract	32.4	-	31.6

% sensitization
● 100%
● 50.0%
○ 0%

** Combination means the total percentage of patients that was sensitized to at least one of the beforementioned tests. This percentage can be lower than one of the percentages of the beforementioned tests due to differences in the total number of patients.*

Patients who underwent oral food challenges with almond

Patient characteristics

In 94 patients, 104 OFCs with almonds were performed, of which 90 with raw almond and 14 with processed almond (10 roasted, 4 blanched). All OFCs with processed almond were negative. All OFCs with processed almond were negative.

Of the 90 patients who underwent an OFC with raw almond, 33.3% were positive (**Table 3**). There were no statistically significant differences in characteristics between the allergic and tolerant patients.

Of the 30 patients with a positive OFC, 36.7% had mild symptoms, 26.7% had moderate symptoms, and 36.7% had severe symptoms (**Table 3 and 4**). All allergic patients had at least subjective symptoms, and 56.7% had also objective symptoms.

Table 3. Characteristics of patients that performed an oral food challenge with almond

	Total (N=90)		**Tolerant (N=60)**		**Allergic (N=30)**		**p-value**
Sex, Female	64	(71.1)	39	(65.0)	25	(83.3)	0.07
Age, mean (SD)	37.0	(14.7)	35.6	(14.5)	39.8	(15.1)	0.21
Level of sIgE/Wheal size							
Level of sIgE, median (IQR)	0.2	(0.0 - 0.7)	0.2	(0.02-0.7)	0.1	(0.1-0.5)	0.49
Wheal size, mean (SD)	4.0	(3.2)	3.7	(3.1)	4.5	(3.9)	0.32
Sensitization							
ImmunoCAP	21	(33.3)	15	(37.5)	6	(26.1)	0.36
SPT	51	(66.2)	32	(61.5)	19	(76.0)	0.21
Severity of reaction OFC							
Mild	11	(12.2)	-		11	(36.7)	-
Moderate	8	(8.9)	-		8	(26.7)	-
Severe	11	(12.2)	-		11	(36.7)	-
Symptoms OFC							-
Subjective	30	(33.3)	-		30	(100.0)	-
Objective	17	(18.9)	-		17	(56.7)	-

All measurements are in N or otherwise specified

Abbreviations: *IQR = Interquartile Range; NA=not applicable; N = Number; OFC = oral food challenge; SD = Standard Deviation; sIgE = specific Immunoglobulin E; SPT = Skin Prick Test*

Table 4. Detailed description of almond-allergic patients

No.	Sex	Age	Subjective / objective	Symptoms	Severity	Subj. threshold value (g)	Obj. threshold value (g)	IgE level (kU_A/L)	SPT wheal size (mm)
1	Female	30	Subjective	OAS, sensation of swelling of tongue and throat	Mild	0.30	-	0.12	-
2	Male	28	Subjective	OAS, sensation of swelling throat	Mild	5.00	-	0.08	4.50
3	Female	37	Subjective	OAS, dysphagia	Mild	10.00	-	0.07	3.00
4	Female	30	Subjective	OAS, itching face	Mild	0.01	-	-	0.00
5	Female	19	Subjective	OAS	Mild	0.30	-	2.99	3.00
6	Female	28	Subjective	OAS	Mild	5.00	-	-	-
7	Female	27	Subjective	OAS	Mild	10.00	-	-	3.00
8	Female	25	Subjective	OAS	Mild	5.00	-	0.27	4.50
9	Male	57	Subjective	Feeling of itching and uncomfortable feeling in chest	Mild	10.00	-	0.90	-
10	Female	31	Objective	OAS	Mild	20.00	20.00	0.00	2.00
11	Female	27	Subjective	OAS, feeling of coughing, feeling of mild dyspnea	Mild	0.01	-	0.22	3.00
12	Female	51	Subjective	OAS, itching hands without erythema, abdominal pain, restlessness, warmth	Moderate	10.00	-	0.05	7.00
13	Female	20	Objective	OAS, rhinitis, itching skin, neck and arms, tearing eyes	Moderate	3.00	20.00	0.19	-
14	Female	42	Objective	OAS, itching eyes, sensation of swelling throat/nose, sneezing, pain in throat	Moderate	0.30	20.00	0.01	5.00
15	Female	23	Objective	OAS, itching cheeks/eyes, rhinitis, nausea, burping	Moderate	0.14	14.29	0.25	3.00
16	Female	63	Objective	OAS, red and itching eyes, peri-orbital swelling	Moderate	10.00	10.00	-	2.00
17	Female	24	Subjective	OAS, itching skin, sensation of swelling throat and wheal upper-lip, nausea	Moderate	1.00	-	0.49	7.50
18	Female	23	Subjective	OAS, sensation of swelling throat, nausea	Moderate	0.30	-	0.05	16.50
19	Female	53	Objective	Feeling of itching eyes/palate/lips, sensation of swelling lips, sneezing, redness/swelling around eyes, red throat, pain swallowing	Moderate	0.10	20.00	0.00	2.50

Table 4. Detailed description of almond-allergic patients (*continued*)

No.	Sex	Age	Subjective / objective	Symptoms	Severity	Subj. threshold value (g)	Obj. threshold value (g)	IgE level (kU_A/L)	SPT wheal size (mm)
20	Female	62	Objective	OAS, nausea, coughing	Severe	0.10	10.00	-	8.00
21	Male	60	Objective	OAS, sensation of swelling throat, bloating, dyspnea with a decrease in peak flow, hoarseness, coughing	Severe	0.14	1.43	0.11	6.00
22	Female	56	Objective	OAS, itching face, itchy eyes, conjunctivitis, mild pressure on chest, severe coughing and local erythema on chest	Severe	0.01	10.00	0.19	-
23	Female	52	Objective	OAS, dysphagia, sensation of swelling throat, running nose, coughing	Severe	0.30	0.30	-	2.00
24	Female	55	Objective	OAS, mucus in throat, dysphagia, itching eyes/whole body, local redness chest, rhinitis, coughing, peri-orbital edema and conjunctivitis	Severe	0.30	10.00	0.47	3.50
25	Male	28	Objective	Feeling of itching fingers/shoulders, dry/numb sensation lips, coughing, dysphagia, rhinitis, mild erythema, transpiration.	Severe	0.10	10.00	6.80	9.50
26	Female	34	Objective	Feeling of itching head, hand palms, dyspnea, erythema face and back, rhinitis	Severe	0.30	1.00	0.73	4.00
27	Female	30	Objective	OAS, dyspnea, nausea, abdominal pain, use of accessory respiratory muscles, decrease in peak flow, expiratory wheezing, coughing	Severe	1.00	1.00	0.00	0.00
28	Female	46	Objective	OAS, itching chest/eyelids, coughing, expiratory wheezing, urticaria left eyelid	Severe	1.00	10.00	-	4.00
29	Male	60	Objective	Itching and erythema, hand palms, swelling eyelids, pressure ears, erythema face and trunk, dyspnea with severe wheezing, peri-orbital edema.	Severe	20.00	20.00	0.04	4.00
30	Female	60	Objective	OAS, itching skin upper body, sensation of swelling throat, coughing, swelling tongue, redness of face, dyspnea, rhinitis, angioedema tongue/throat, dyspnea, erythema	Severe	0.01	0.30	0.06	4.00

The correlation between almond-sIgE levels and wheal sizes was 0.36 (p=0.006). Although not statistically significant, there were more tolerant (37.5%) than allergic (26.1%) patients sensitized to almond extract by ImmunoCAP (**Figure 2A**). Regarding the patients with a positive OFC result, there was also no statistically significant difference between patients with only subjective and also objective symptoms regarding almond extract in ImmunoCAP and SPT (**Figure 2B**).

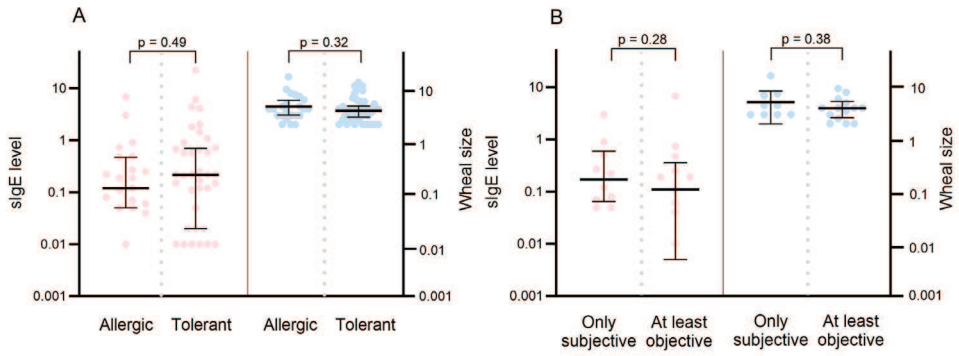


Figure 2. Almond-sIgE levels with medians and interquartile ranges and wheal sizes with means and standard deviations in patients with OFC with raw almonds for patients **A** who were allergic or tolerant and **B** who had only subjective symptoms or also objective symptoms when OFC was positive

Diagnostic value

The discriminative ability for predicting almond allergy was both low for almond-sIgE (AUC of 0.54 [95% CI = 0.38 – 0.71]) and SPT (AUC of 0.64 [95% CI = 0.49– 0.80]). The combination of both tests was somewhat better, with an AUC of 0.68 (95% CI = 0.53 - 0.83) (**Figure 3A**). When predicting objective symptoms, only the discriminative ability for sIgE slightly improved (AUC 0.64 [95% CI 0.38-0.89]), but this was still too low to use in clinical practice (**Figure 3B)**.

The sensitivity, specificity, PPV, and NPV of almond-sIgE and SPT, and their combination at the clinical and optimal cutoff point are presented in **Table 5**. For sIgE to almond extract, no value was good enough for use in clinical practice. For SPT with almond extract, the sensitivity and NPV of 76.0% and 76.92%, respectively, were acceptable to rule out an almond allergy.

Using the optimised cutt-off (using the Youdens' index) for sIgE to almond extract did not increase the diagnostic value. For SPT with almond extract this did increase the sensitivity and slightly increased the NPV, but with a wheal size cut-off point of 0.31 mm, many patients with a negative almond allergy would be false positives. The combination of almond-sIgE and SPT performed best, but the specificity and PPV were still low.

It was not possible to calculate the 100% and 95% PPV and NPV because the almond-sIgE levels and wheal sizes did not discriminate well enough between tolerance and allergy, with high and low values in both groups.

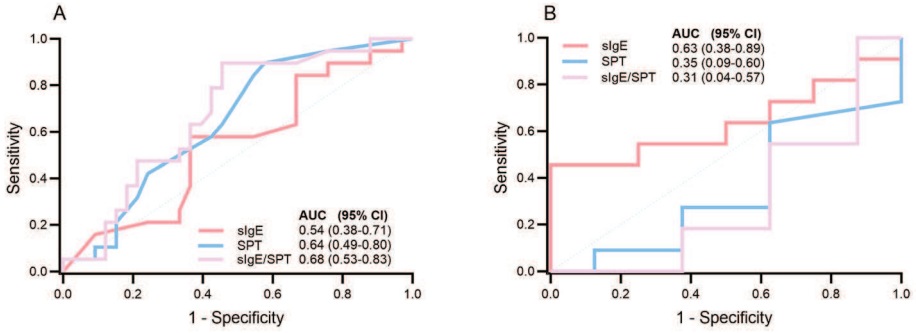


Figure 3. ROC curves of almond-sIgE and SPT and the combined diagnostic tests in patients with a positive or negative OFC (**3A**) and in patients that had only subjective or also objective symptoms during OFC (**3B**)
Abbriviations: AUC = area under the curve, CI = confidence interval

Table 5. Sensitivity, specificity, positive predictive value and negative predictive value of almond-sIgE and SPT, and their combination at the clinical and optimal cutoff point

	sIgE	Youden sIgE	SPT	Youden SPT	Youden sIgE + SPT
Cut-off value	0.35	0.37	3.00	0.31	0.33
Sensitivity (95% CI)	26.09 (10.23-48.41)	26.09 (10.23-48.41)	76.00 (54.87-90.64)	92.00 (73.97-99.02)	89.47 (66.86-98.70)
Specificity (95% CI)	62.50 (45.80-77.27)	62.50 (45.80-77.27)	38.46 (25.30-52.98)	18.37 (8.67-32.02)	39.39 (22.91-57.86)
Postive predictive value (95% CI)	28.57 (15.29-46.99)	28.57 (15.29-46.99)	37.25 (30.39-44.68)	36.51 (32.53-40.68)	45.95 (38.28-53.81)
Negative predictive value (95% CI)	59.52 (51.11-67.42)	59.52 (51.11-67.42)	76.92 (60.50-87.89)	81.82 (51.24-95.07)	86.67 (62.11-96.27)

DISCUSSION

To our knowledge, this is the first paper that describes the clinical characteristics, extensive sensitization patterns, coallergies and severity of patients with a (suspected) almond allergy including the diagnostic value of routinely used almond-sIgE and SPT in an adult population. With one third of the patients being positive to raw almond during OFC, we showed that almond allergy in adults is not as rare as earlier reported in children.[16] Furthermore, we also showed that almond allergy is severe in 29.1% of patients with suspected almond allergy and 36.7% of patients with a positive OFC. Reported symptoms to

hazelnut and walnut were common, whereas symptoms to other nuts were uncommon. Finally, both almond-sIgE and SPT are not useful for diagnosis in clinical practice, with an AUC of 0.54 and 0.64, respectively.

More positive OFC to almonds in adults than in children

Our study showed that one third of the OFCs were positive. This rate is much higher than previously reported: four studies showed a very low positive OFC rate between 1.2-5.0%, which were mainly performed in children.[5,6,8,10] It could be that different criteria were used to define a positive OFC. In our study, an OFC was considered positive if subjective symptoms persisted for at least 45 minutes or when objective allergic reactions occurred. Some studies considered an OFC positive only when objective symptoms occured[8] or excluded patients experiencing only oral symptoms[10]. However, even if we excluded the 13 patients in our study with only subjective symptoms, 18.9% of all OFCs were still positive. So, a positive almond OFC seems not that rare in adults as previously reported in children.

Another reason for this disparity could be that the a priori probability of a positive OFC to almonds in other studies was lower than in our study. Of the patients who underwent an OFC in our study, 30% had self-reported symptoms prior to OFC, and of these, 48.1% had a positive challenge (data not shown). Virkud et al. showed that only 11% of the patients had self-reported symptoms and similar proportion (45%) had a positive OFC.[6] Nearly 90% of patients, mostly children, who underwent an OFC reported no symptoms of almonds prior to OFC, which therefore might result in a lower a priori risk of almond allergy.

Almond allergy is severe in one third of the patients

We showed that almond allergy can be severe, as demonstrated by 29.1% of patients with suspected almond allergy reporting severe symptoms and 36.7% of patients with a positive almond OFC having a severe reaction. Quite similar results were found in three other studies: at first glance, Virkud et al. showed that only 4% had Grade 2/3 symptoms and 0.5% had anaphylaxis in all performed almond OFCs.[6] However, when only taking into account the positive challenges in this study, 60% had a Grade 2 reaction and 10% a Grade 3 reaction. Andorf et al. also showed that 10% of the patients with a positive OFC had severe symptoms (pulmonary symptoms were not further specified).[4] Baker and Kattan showed a table of all symptoms reported during the challenge per patient. When these symptoms were graded using the oFASS-3 scoring, 31.2% had severe symptoms.[7] Taken together, these studies show that 10-30% of mostly children with a confirmed almond allergy have a severe allergy, which is in line with our findings in adults.

One study of Pouessel et al. showed a much lower rate of severity: of the 189 children who underwent a challenge, only 1.6% were positive, all of which were mild.[5] This difference might be explained by a different form of processing of almond used during OFCs. Pouessel et al. did not document the type of processing that was used during

OFC, whereas most of our OFCs were performed with raw almonds. It is very likely that Pouessel et al. used at least pasteurized almonds, as nearly 70% of all almonds sold in the European market come from California where it is mandatory to pasteurize almonds prior to shipment to reduce the risk of Salmonella.[11,17,18] Thermal processing may strongly reduce the allergenicity of almonds[12]. This was observed in our study where 10 patients underwent an OFC for both processed and raw almond, of whom all the processed OFCs were negative, whereas 50% of the raw OFCs were positive.

Co-allergies and co-sensitization to hazelnut, apple, and walnut are common in almond allergic patients

This is one of the first studies that investigated in detail co-allergies and co-senstization in almond allergic patients. Patients who reported allergic symptoms to almond most often also reported symptoms to hazelnut, apple, and walnut, which is probably caused by cross-sensitation with the birch pollen PR-10 protein Bet v 1 and its homologues in food.[19] This is confirmed by the fact that most patients were also sensitized to hazelnut and the PR-10 proteins (Gly m 4, Cor a 1, and Ara h 8). The lower cosensitisation to walnut can be explained by the low concentration of the PR-10 protein Jug r 5 in walnut extract.[20] Co-allergy and cosensitisation to other nuts was low. Other studies showed unclear or comparable results. The two studies that provided information on the co-alllergies included too few almond allergic patients to draw conclusions [10,21] and three studies showed the highest cosenisitization between almond and hazelnut.[10,22,23]

As our study showed that most of the patients who reported symptoms to almonds also reported symptoms to hazelnut, and even more patients were sensitized to Cor a 1 than to almond extract, we hypothesized that Cor a 1 might be a better predictor of almond allergy than almond-sIgE and SPT in the absence of the ability to use almond components. A post-hoc analysis (**Figure S1**) confirmed our hypothesis: Cor a 1 performed better with an AUC of 0.71, but this value was still deemed too low to use in clinical practice. It would be interesting to carry out further research into the diagnostic value of components in predicting almond allergy.

Almond-sIgE and SPT not useful to predict almond allergy

The discriminative ability for predicting almond allergy was low, with AUCs of 0.54 for sIgE, 0.64 for SPT, and 0.68 for the combination of both tests.

Virkud et al. showed comparable results with AUCs of 0.61 for sIgE, 0.66 for SPT and 0.67 for the combination of both.[6] Three other studies showed that there was no statistically significant difference between a positive and negative OFC with almond and sensitization to SPT or sIgE to almond extract, further illustrating the poor diagnostic value.[4,5,7] Only one study showed a better discriminative ability to predict almond allergy with an AUC of 0.87 for sIgE to almond extract. However, they also showed that the estimated 95%

predicted probability of a positive OFC would have a cut-off value of 167 kU_A/L which is much higher than any of the observed almond sIgE results in their population.[8]

Component resolved diagnostics (CRD) offers the potential to increase the accuracy of allergy diagnosis.[24] In adults, multiple studies have shown a higher diagnostic accuracy for a component than for the extract, such as peanut[25] and cashew nut[26]. At this moment, 7 almond components have been chacterized [27], but currently, none is commercially available for clinical diagnostics. Further development and validation of these components would be essential to make them available for clinical use.

Strengths and limitations

The main limitation of this study was that we did not perform an OFC in all patients, although we did perform 114 OFCs in 94 patients. Furthermore, it would be insightful to perform OFCs with processed and raw almond in all patients. Another limitation is that our study was performed in a tertiary center situated in a birch-endemic area. Therefore, it could be that the results are not generalizable to other centers/countries.

The strength of this study is that this is the first that investigated almond allergy in a well-defined adult population. We did not only investigated patients with reported symptoms to almond, but also patients who performed OFCs with almond. This large group of adult patients allowed us to evaluate in detail the clinical characteristics and diagnostic value of almond-sIgE and SPT.

In conclusion, almond allergy to raw almonds is severe in a substantial part (36.7%) of the patients, while all OFCs with processed almond were negative. Therefore, when (severe) symptoms to raw almond are reported, this does not imply a reaction to processed almond. OFCs with only processed almond may miss a potentially severe allergic reaction to raw almond, and doctors should consider performing an OFC with raw almond as well to give patients the best dietary advise. Both almond-sIgE and SPT are of limited use in clinical practice for predicting almond allergy. There is a need for better diagnostic tests that can predict presence and severity of almond allergy.

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SUPPLEMENTAL

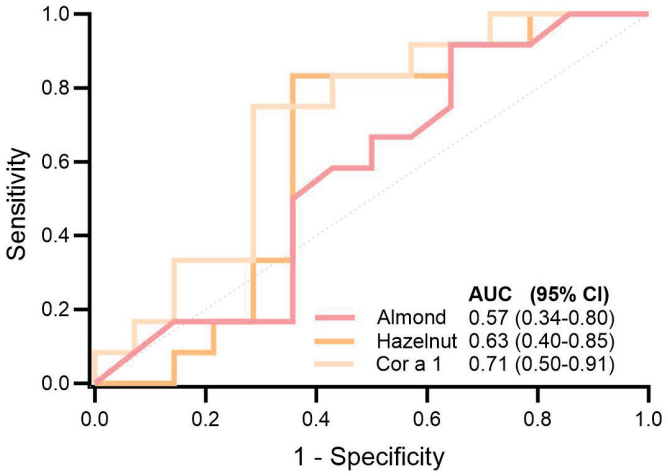


Figure S1. ROC curves of sIgE to almond extract, hazelnut extract and Cor a 1 in in patients with a positive or negative OFC with almond
Abbriviations: AUC = area under the curve, CI

5

Sensitization patterns and clinical value of six almond components in two European cohorts – Netherlands vs Portugal

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Under review

Background: Component resolved diagnostics (CRD) in almond allergy have not been studied comprehensively. Sensitization to six almond components was compared between The Netherlands (NL) and Portugal (PT), and potential implications for primary sensitization sources and diagnosis were explored.

Methods: Adults with suspected almond allergy from two centers underwent specific IgE testing to almond extract and six recombinant components: Pru du 1 (PR-10), Pru du 3 (long and short) (LTP), Pru du 6 (11S globulin), Pru du γ-conglutin (7S globulin), and Pru du 2S albumin. Allergens were produced in *E.coli* using SUMO expression, purified, biotinylated, and coupled to streptavidin-coated ImmunoCAPs. Sensitization patterns were compared between countries. Severity predictors were analyzed in 122 patients with probable almond allergy using logistic regression.

Results: Among 130 patients, Pru du 1 sensitization was more frequent in NL (82.9% vs 55.0%), whereas Pru du 3 sensitization was more common in PT (66.7% vs 22.9%). Among seed storage proteins, Pru du γ-conglutin was most common (12.3%), followed by Pru du 6 (3.9%); IgE to Pru du 2S was virtually absent. Correlations between Pru du 1 and Bet v 1 were stronger in NL compared to PT, while Pru du 3 and Pru p 3 showed the opposite trend. Component-specific IgE did not distinguish allergic from tolerant individuals or predict severity. PR-10 sensitization and older age were associated with milder symptoms, especially in PT.

Conclusions: Almond CRD revealed clear geographic differences in PR-10 and LTP sensitization. The near-absence of IgE to 2S albumins was notable. CRD combined with demographic factors may support risk stratification.

INTRODUCTION

Almond allergy is a clinically relevant food allergy in Europe, with adult prevalence estimates ranging from 0.2% to 4.1%, depending on region.[1,2] Accurate, accessible diagnostic tools are essential. Skin prick testing (SPT) and serum specific IgE (sIgE) to almond extract are recommended first-line tools in almond allergy diagnosis.[3] Component-resolved diagnostics (CRD) have significantly improved characterization and diagnosis of several tree nut allergies, including cashew, pistachio and hazelnut.[4–7] In contrast, primary sensitizers and clinically relevant components in almond allergy are less well defined, and it is unknown whether CRD adds diagnostic or prognostic value beyond extract-based testing. To address this, a prerequisite is to establish a comprehensive panel of almond allergens.

Currently, seven almond allergen components (*Prunus dulcis*) are officially recognized by the WHO-IUIS Allergen Nomenclature Subcommittee: Pru du 1 (PR-10), Pru du 3 (non-specific lipid transfer protein [nsLTP]), Pru du 4 (profilin), Pru du 5 (60S acidic ribosomal protein P2), Pru du 6 (amandin; 11S globulin), Pru du 8 (α-hairpinin), and Pru du 10 (mandelonitrile lyase 2).[8–15] Additional allergens have been described, but supporting data are limited and sometimes debated. Based on genome sequence homology with thaumatin-like protein (TLP) allergens from peach and apple, almond TLP was proposed as an allergen, but without IgE-binding evidence.[16] Pru du 2S albumin (prolamin seed storage protein) was also considered, based on IgE-binding to short peptides with partial homology to tree nut 2S albumins, but these peptides are now thought to be related to the hairpinin Pru du 8.[14] Conversely, Pru du γ-conglutin (vicilin-like 7S globulin) has shown more convincing evidence as an almond allergen.[15]

While sensitization patterns to almond allergen components across Europe are lacking, they have been well-characterized for hazelnut and walnut.[17,18] Pan-European studies have shown a similar pattern: sensitization to PR-10 proteins is more prevalent in Northern and Central Europe and LTP sensitization predominates in Mediterranean areas. Sensitization to PR-10 food proteins is thought to follow primary sensitization to birch pollen Bet v 1 (PR-10)[19], while LTP sensitization has been linked to peach Pru p 3 as primary driver in Southern Europe.[20]

CRD has been reported to improve prediction of food challenge outcomes compared with extract-based sIgE for several tree nuts, with 2S albumins showing particularly good performance as markers of clinical allergy.[21] Evidence on the diagnostic utility of almond components is limited. One study identified Pru du 6 as a potential marker of almond allergy, with a reported sensitivity of 83% and specificity of 78%.[22] Another study suggested that Pru du 1 may be underrepresented in almond extract, leading to poor correlation with extract-sIgE and possible false-negative results.[23] These findings highlight

the potential value of incorporating component-level diagnostics into almond allergy assessment.

CRD may also inform reaction severity.[4,5] Sensitization to stable proteins – such as nsLTPs and seed storage proteins (SSPs), typically resistant to heat and digestion – has been associated with systemic and more severe reactions.[24] In contrast, sensitization to labile proteins, such as PR-10, is generally linked to milder, localized symptoms, although severe reactions can occur.[25,26] To date, no studies have systematically evaluated the relationship between sensitization to almond components, different geographical regions and clinical severity.

The aims of this study were to: (1) compare sensitization patterns to the six almond components in two distinct regions – birch-endemic The Netherlands and Southern European Portugal; (2) compare likely sources of primary sensitization in both areas, and (3) explore whether almond CRD can help distinguish tolerant from allergic individuals and predict severity.

METHODS

Study population

Patients aged ≥ 17 years were retrospectively included between 2012 and 2024 from the Department of Dermatology and Allergology, University Medical Center Utrecht (UMCU), The Netherlands (NL), and the Department of Allergy and Clinical Immunology, Coimbra University Hospital (ULS Coimbra), Portugal (PT). Eligible patients had almond-related symptoms, reported or documented by a healthcare provider – possible almond allergy. All patients were assessed for sensitization to almond extract using specific IgE (ImmunoCAP), and a subgroup underwent an oral food challenge (OFC). Patients were excluded if available serum volume was insufficient for sIgE determination.

The study was approved by the local medical ethics committees (protocols B23-233 and OBS_SF_229_2022).

Almond component protein synthesis and ImmunoCAP conjugation

Six recombinant almond allergens, based on the *Nonpareil* almond genome[27] – Pru du 1 (PR-10), Pru du 3 long and short (nsLTPs), Pru du 6 (amandin/11S globulin), Pru du γ-conglutin (vicilin-like 7S globulin), and Pru du 2S albumin – were produced in *E. coli* and purified using a standardized SUMO-based expression system.

Proteins were biotinylated and coupled to streptavidin ImmunoCAPs (Ro212, Thermo Fisher Scientific, Uppsala, Sweden) at 1.0 µg per test. A recombinant SUMO control was prepared for background correction.

The translated almond protein sequences are depicted in **Figure S1**. Full methodological details, including sequences and expression/purification steps, are provided in **Supplemental 1**.

Data collection

Clinical and demographic data

Clinical and demographic data, including age, sex, allergic comorbidities (allergic rhinitis, allergic asthma, atopic dermatitis), skin prick test (SPT) results and OFC outcomes, were extracted from medical records.

Reaction severity to almond was classified using the ordinal Food Allergy Severity Score (oFASS-5) scale.[28] This validated system classifies food allergic reactions into five grades based on the organ systems involved. Grade 1 symptoms involved reactions restricted to the oral cavity; Grade 2 symptoms involved a single organ/system such as skin, eye/nose, digestive tract, or uterus, with or without grade 1 symptoms; Grade 3 involved more than one of grade 2 symptoms, with or without grade 1; Grade 4 involved symptoms of the larynx and/or bronchi with or without grade 1-3 symptoms; Grade 5 involved symptoms of the cardiovascular and/or nervous system with or without grade 1-4 symptoms.

Grade 1 reactions were considered mild, Grades 2 or 3 represented moderate symptoms, and Grades 4 or 5 severe reactions.

Sensitization tests

Sensitization to almond extract was assessed by SPT (ALK, Madrid, Spain), prick-to-prick testing (PTP) with raw almond, and by measurement of sIgE on ImmunoCAP. In all patients, sIgE was measured against the six recombinant components: rPru du 1, rPru du 2S albumin, rPru du 3, rPru du 6, and rPru du γ-conglutin. In addition, sIgE levels for birch pollen rBet v 1 and peach rPru p 3 were measured. Sensitization was defined as a mean wheal diameter ≥3 mm (SPT or PTP) or sIgE ≥ 0.10 kU_A/L. For those allergens expressed in the SUMO expression platform, a control test was performed with "naked" SUMO-peptide conjugated ImmunoCAPs.

sIgE data for additional potentially cross-reactive PR-10s, LTPs, and SSPs from other tree nuts and peanut measured by ImmunoCAP were retrospectively collected (**Figure S2**), and are described in **Supplemental 2**.

Oral food challenge

Open OFCs with raw almond were performed according to center-specific PRACTALL-based protocols.[29] Challenges used stepwise incremental dosing or a single-dose protocol depending on local practice.

A challenge was considered positive when objective signs occurred or when persistent subjective symptoms (≥ 45 minutes) were observed.

Full dosing schemes are provided in the **Supplemental 3**.

Definitions of almond allergy

Almond allergy was categorized as follows:

- Possible allergy**:** symptoms suggestive of an allergic reaction to almond within two hours of ingestion, evaluated by a clinician using a standardized questionnaire (adapted from the EuroPrevall studies), but without confirmation of sensitization.[3,30]
- Probable allergy: symptoms within two hours of ingestion, combined with sensitization to almond extract (SPT/PTP/sIgE) and/or components.
- Confirmed allergy: positive open OFC to raw almond.

Statistical analysis

Almond sensitization patterns across Portugal and the Netherlands

Continuous variables (e.g., age) were summarized as mean ± standard deviation (SD) when normally distributed or as median with interquartile range (IQR) when non-normally distributed. Categorical variables (e.g., sex) were summarized as frequencies and percentages.

Between-country comparisons were performed using independent t-tests for normally distributed continuous variables, Mann-Whitney U tests for non-normally distributed variables, and Pearson chi-square or Fisher's exact tests for categorical variables.

Correlations between sIgE to almond components and homologous allergens were assessed using Spearman's rank correlation coefficient (ρ).

Predicting severity of almond allergy

Severity analyses were limited to patients with probable almond allergy. Symptom severity was categorized as mild or moderate to severe to balance group sizes and to account for the high proportion of patients presenting with oral allergy syndrome (OAS), while differentiating it from mucocutaneous or systemic reactions.

Univariable logistic regression examined associations between severity and demographic variables, atopic comorbidities, sensitization measures and OFC outcomes. Variables with $p < 0.20$ in univariable analysis or deemed clinically relevant (sensitization to almond extract and its components, due to their biological plausibility as predictors) were entered into multivariable models using stepwise backward selection.

Subgroup analyses were conducted separately for the Dutch and Portuguese cohorts. Model discrimination was evaluated using AUC-ROC with 95% confidence intervals. Additionally, an exploratory analysis evaluated the ability of almond extract-based and component sIgE to distinguish OFC-allergic from OFC-tolerant patients.

All analyses were performed using IBM SPSS Statistics (version 29.0.1) and MedCalc Statistical Software (version 23.0.9).

RESULTS

Characterization of 6 recombinant almond components

All six recombinant almond proteins were successfully expressed and purified (**Figure 1**). Pru du 1 (PR-10) was identified as a new variant (Pru du 1.0102) with approximately 75% sequence identity to Pru du 1.0101. The long version of Pru du 3 long represented a novel isoallergen (Pru du 3.0201), with < 60% amino acid identity to Pru du 3.0101 but conserved positioning of 8 cysteine residues common to LTPs. The short version represents a novel variant (Pru du 3. 0102) of Pru du 3.0101, with a single amino acid change (T→S) at position 92. Pru du γ-conglutin, a vicilin-like 7S globulin, showed sequence similarity to other 7S globulins such as Ara h 1 but is not yet listed as an official almond allergen. Pru du 6 (11S globulin) was a new variant (Pru du 6.0102) of Pru du 6.0101 (> 99.4% identity), differing at three positions. Lastly, Pru du 2S albumin displayed the common 8 cysteine signature shared with other tree nut and peanut 2S albumins, such as Cor a 14 and Ara h 2.

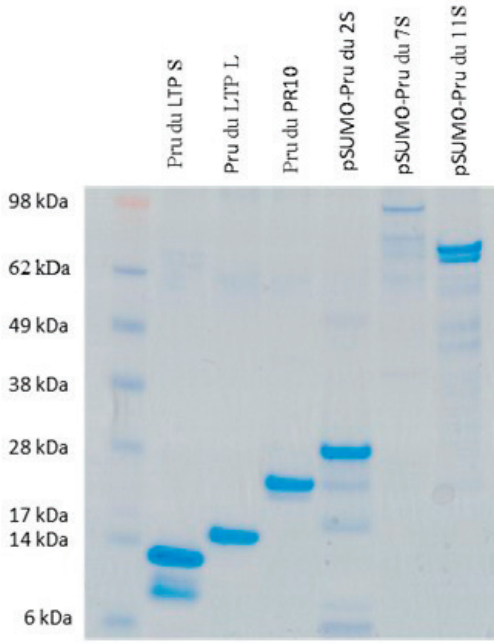


Figure 1. SDS-Page of recombinant almond allergens
Left to right: Pru du 3 (short and long), Pru du 1, Pru du 2S albumin, Pru du y-conglutin and Pru du 6.

Patient characteristics

A total of 130 patients were included in this study of whom 30.0% were male (**Table 1**). Allergic rhinitis was the most prevalent atopic comorbidity (89.6%), followed by atopic dermatitis (51.5%) and asthma (50.4%). Birch pollen allergy was significantly more common in NL (80.6% vs 29.6%, *p<0.001*), while grass pollen allergy was more common in PT (74.4% vs 40.0%, *p=0.001*).

Although most patients reported mild symptoms (43.8% oFASS-5 grade 1), still 20.0% of the patients reported severe symptoms (17.7% oFASS-5 grade 4; 2.3% oFASS-5 grade 5). There were no significant differences in self-reported symptom severity between patients from NL and PT.

According to the study's definitions, 122 (93.8%) patients met criteria for probable almond allergy, and 18 (13.8%) for confirmed almond allergy.

Twenty-four patients underwent OFC, of whom 75.0% reacted **(Table 1)**. Most challenges were performed in NL (70.8%), but proportion of positive challenges was similar between countries (70.6% vs. 85.7%, p=0.629). Almond extract and individual components showed low sensitivity and specificity, with no clinically meaningful discrimination between OFC-positive and OFC-negative patients.

Almond sensitization patterns across the Netherlands and Portugal

The distribution of sensitization to almond extract and specific almond components is presented in **Table 1** and **Figure 2**. Overall, sensitization to almond extract was highly frequent (82.3%). Among components, sensitization to the PR-10 protein Pru du 1 was most common (70.0%), followed by Pru du 3 long (43.1%) and short (37.7%), Pru du γ-conglutin (12.3%) and Pru du 6 (3.9%). After subtraction of background SUMO reactivity, sIgE to Pru du 2S albumin was virtually non-existent (2.3%), with three borderline positive results (< 0.60 kU_A/L). In the light of the background IgE-binding to the SUMO3-tag, these positive responses need to be viewed with caution.

Significantly more patients were sensitized to Pru du 1 in Utrecht compared to Coimbra (82.9% *vs* 55.0%), whereas sensitization to Pru du 3 isoforms (long and short) was more frequent in Coimbra compared to Utrecht (66.7% *vs.* 22.9% for the long isoform). Median sIgE levels also reflected this pattern: NL patients had higher median levels to Pru du 1 compared to the PT cohort (3.26 vs. 0.24 kU_A/L), whereas the opposite was observed for Pru du 3 (0.04 vs. 1.03 kU_A/L for the long isoform).

Table 1. Baseline clinical and immunological characteristics from Utrecht (NL) and Coimbra (PT) centers.

Variables	Total (n=130)	*Max*	Utrecht (n=70)	*Max*	Coimbra (n=60)	*Max*	*p-value*
Demographics							
Age (y), mean ± SD	36.2 ± 14.6	130	34.2 ± 12.0	70	38.4 ± 17.0	60	*0.364*
Male sex	39 (30.0)	130	20 (28.6)	70	19 (31.7)	60	*0.701*
Atopic comorbidities							
Allergic rhinitis	112 (89.6)	125	62 (88.6)	70	50 (90.9)	55	*0.671*
Probable birch pollen allergy	70 (60.9)	106	50 (80.6)	62	13 (29.6)	44	***<.001***
Probable grass pollen allergy	50 (56.8)	88	18 (40.0)	45	32 (74.4)	43	***0.001***
Atopic dermatitis	67 (51.5)	130	41 (58.6)	70	26 (43.3)	60	*0.085*
Allergic asthma	63 (50.4)	125	33 (47.1)	70	30 (54.6)	55	*0.939*
Symptom severity (self-reported)							
oFASS-5 grade 1	57 (43.8)	130	30 (42.9)	70	27 (45.0)	60	*0.337*
oFASS-5 grade 2	37 (28.5)	130	18 (25.7)	70	19 (31.7)	60	
oFASS-5 grade 3	10 (7.7)	130	3 (4.3)	70	7 (11.7)	60	
oFASS-5 grade 4	23 (17.7)	130	16 (22.9)	70	7 (11.7)	60	
oFASS-5 grade 5	3 (2.3)	130	3 (4.3)	70	0 (0.0)	60	
sIgE levels, median (Q1-Q3)							
Almond extract	0.49 (0.17-1.27)	130	0.44 (0.17-1.10)	70	0.53 (0.17-1.96)	60	*0.460*
Pru du 1	1.26 (0.04-5.09)	130	3.26 (0.58-7.50)	70	0.24 (0.02-1.56)	60	***<.001***
Pru du 3 long	0.07 (0.01-0.85)	130	0.04 (0.00-0.09)	70	1.03 (0.03-6.00)	60	***<.001***

Table 1. Baseline clinical and immunological characteristics from Utrecht (NL) and Coimbra (PT) centers. (*continued*)

Variables	Total (n=130)	*Max*	Utrecht (n=70)	*Max*	Coimbra (n=60)	*Max*	*p-value*
Pru du 3 short	0.05 (0.01-0.50)	130	0.02 (0.01-0.06)	70	0.55 (0.02-6.64)	60	***<.001***
Pru du 2S albumin*	0.00 (0.00-0.02)	130	0.00 (0.00-0.01)	70	0.01 (0.00-0.02)	60	***<.001***
IgE Pru du y-conglutin*	0.01 (0.00-0.04)	130	0.01 (0.00-0.07)	70	0.01 (0.00-0.03)	60	*0.418*
IgE Pru du 6*	0.01 (0.00-0.02)	130	0.00 (0.00-0.02)	70	0.01 (0.00-0.02)	60	*0.264*
Sensitization to almond							
SPT almond extract	63 (63.6)	99	42 (75.0)	56	21 (48.8)	43	***0.007***
PTP almond extract	32 (68.1)	47	1 (25.0)	4	31 (72.1)	43	*0.089*
IgE almond extract	107 (82.3)	130	57 (81.4)	70	50 (83.3)	60	*0.777*
IgE Pru du 1	91 (70.0)	130	58 (82.9)	70	33 (55.0)	60	***<.001***
IgE Pru du 3 long	56 (43.1)	130	16 (22.9)	70	40 (66.7)	60	***<.001***
IgE Pru du 3 short	49 (37.7)	130	11 (15.7)	70	38 (63.3)	60	***<.001***
IgE Pru du 2S albumin*	3 (2.3)	130	0 (0.0)	70	3 (5.0)	60	*0.096*
IgE Pru du y-conglutin*	16 (12.3)	130	12 (17.1)	70	4 (6.7)	60	*0.107*
IgE Pru du 6*	5 (3.9)	130	4 (5.7)	70	1 (1.7)	60	*0.373*
Sensitization to other allergens							
Birch Bet v 1	84 (64.6)	130	61 (87.1)	70	23 (38.3)	60	***<.001***
Peach Pru p 3	64 (49.2)	130	21 (32.8)	70	43 (71.7)	60	***<.001***
Oral food challenge result							
Negative	6 (25.0)	24	5 (29.4)	17	1 (14.3)	7	*0.629*
Positive	18 (75.0)	24	12 (70.6)	17	6 (85.7)	7	
Symptom severity (oral food challenge)							
oFASS-5 grade 1	6 (33.3)	18	3 (25.0)	12	3 (50.0)	6	*0.062*
oFASS-5 grade 2	5 (27.8)	18	2 (16.7)	12	3 (50.0)	6	
oFASS-5 grade 3	2 (11.1)	18	2 (16.7)	12	0 (0.0)	6	
oFASS-5 grade 4	5 (27.8)	18	5 (41.6)	12	0 (0.0)	6	
oFASS-5 grade 5	0 (0.0)	18	0 (0.0)	12	0 (0.0)	6	

All measurements are in N (%) unless otherwise specified. In case values were missing, percentage in brackets is related to total number of available results. Statistically significant comparisons ($p < 0.05$) are highlighted in bold.

*Abbreviations: SD, standard deviation; sIgE, specific immunoglobulin E; SPT, skin prick test; PTP, prick-to-prick test; oFASS, oral Food Allergy Severity Score; OFC, oral food challenge; w/o, without. *- levels adjusted for SUMO3 subtraction.*

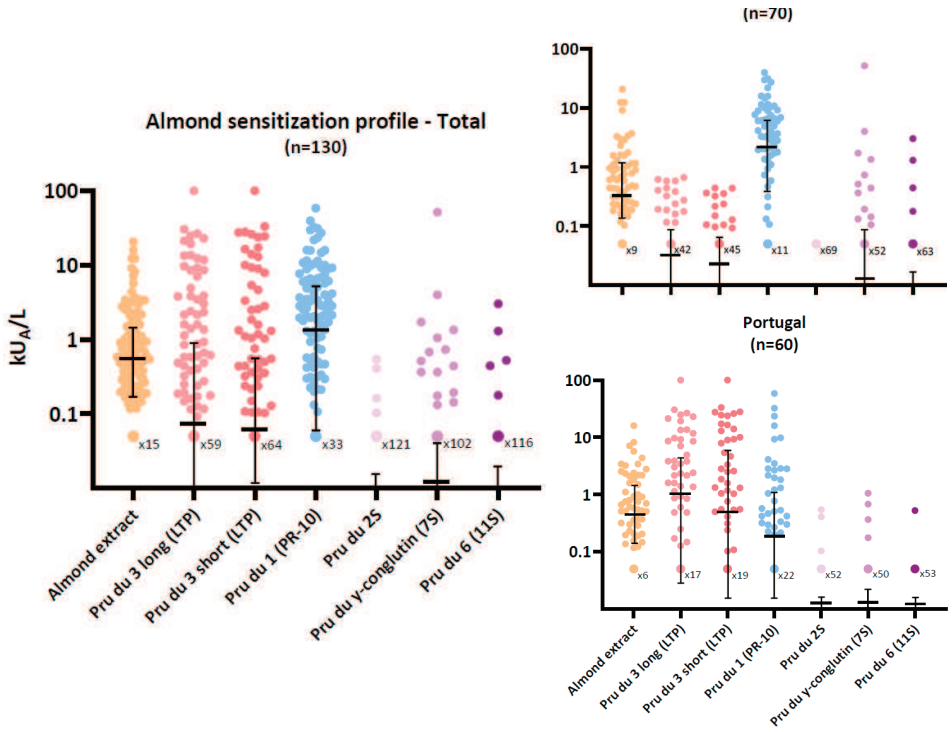


Figure 2. Distribution of almond-specific IgE levels in the total population and by country
Black bars represent median and interquartile range. xN represents the number of measurements <0.05 kU_A.

Sensitization to almond extract and components showed substantial overlap, yet some discordance was noted. Among the 23 patients (17.7%) not sensitized to almond extract, 10 (43.5%) were sensitized to Pru du 1 and two to Pru du 3 isoforms, whereas none were sensitized to almond SSPs. The mean ratio of sIgE sensitization to Pru du 1 over that to almond extract was 3.1, indicating that the sensitivity of the almond extract for picking up sIgE against Pru du 1 is sub-optimal.

Correlations between Pru du 3 long and short sIgE were strong (ρ=0.86). Both isoforms correlated closely with peach Pru p 3 (ρ=0.77 and 0.82, respectively), and Pru du 1 correlated strongly with Bet v 1 (ρ=0.84). Stratified analyses revealed a stronger correlation between Pru du 1 and Bet v 1 in Dutch patients than in Portuguese patients (ρ=0.87 vs 0.60; **Figure 3**), supporting birch pollen as the predominant PR-10 primary sensitizer in NL, whereas other tree pollens likely contribute in PT**.** Although hazel pollen exposure is not very high in Coimbra, correlations between Pru du 1 and Cor a 1 were high in both cohorts (ρ=0.92 vs. 0.90). Pru du 3 correlations with Pru p 3 showed the opposite trend, with markedly stronger correlations in PT vs. NL (Pru du 3 long-Pru p 3: ρ=0.93 vs. 0.44), suggesting that the paradigm of peach being the primary LTP sensitizer does not hold true for a non-Mediterranean country **(Figure 3).**

Correlations between SSPs were also assessed (**Figure S2)**. Due to limited number of borderline positive responses, correlation analysis for Pru du 2S albumin was not pursued. For 7S globulins, Pru du γ-conglutin showed a moderate correlation with Ara h 1 sIgE levels in the Portuguese cohort (ρ=0.55). For 11S globulins, Pru du 6 correlated weakly with Cor a 9 in the overall cohort (ρ=0.28) and in the Dutch subgroup (ρ=0.26), suggesting that there may be some degree of cross-reactivity.

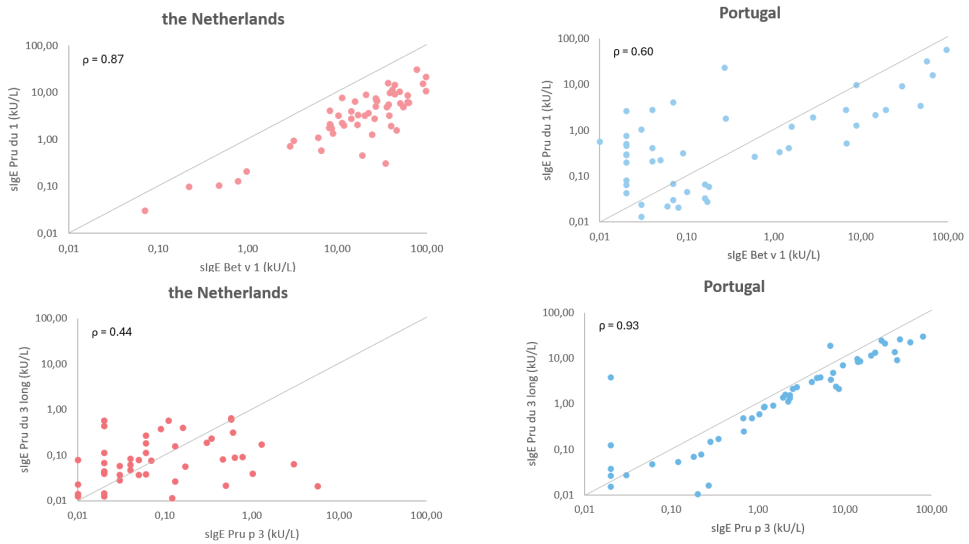


Figure 3. Comparison of IgE reactivity between almond components and homologous allergens in the Netherlands and Portugal

Top: Pru du 1 vs Bet v 1 (PR-10), showing stronger correlation in the Netherlands (ρ=0.87) than in Portugal (ρ=0.60). **Bottom:** Pru du 3 long vs Pru p 3 (nsLTP), with weaker correlation in the Netherlands (ρ=0.44) and stronger correlation in Portugal (ρ=0.93). Dashed lines indicate the line of identity; boxes mark the 0.10 kU_A/L threshold region.

Predictors for severity of almond allergy

Among the 122 individuals with probable almond allergy, 44.3% reported mild reactions (oFASS-5 grade 1), while 55.7% experienced moderate-to-severe reactions (grades 2-5). A detailed summary of univariate comparisons is provided in **Table 2**. Pru du 1 and Bet v 1 sensitization, as well as probable birch pollen allergy, were associated with milder reactions in the total cohort, driven mainly by the PT subgroup. There was a trend for sensitization to the short version of Pru du 3 to be associated with moderate-severe reactions in the whole population and the NL cohort. No significant associations were found between severity and sIgE levels to almond extract or individual components. However, a trend toward higher sIgE levels to Pru du 1 in patients with milder symptoms was observed, especially in Portugal. Additionally, elevated median Bet v 1 levels were associated with milder reactions (14.44 vs. 1.88 kU/L, *p=0.042*).

In multivariable analysis of the combined cohort, a logistic regression model including sIgE to all six almond components alone showed poor discrimination (AUC 0.60; 95% CI: 0.50-0.70). In contrast, an alternative model including Pru du 1 and Pru du γ-conglutin sensitization, almond SPT sensitization and age improved performance (AUC 0.71; 95% CI: 0.61-0.81). Within this model, Pru du 1 sensitization (OR 0.36; *p=0.080*) showed a non-significant trend toward a protective effect, whereas Pru du y-conglutin sensitization (OR 3.40; *p=0.091*) showed a non-significant trend towards increased severity. Positive SPT to almond extract (OR 2.97; *p=0.035*) was a predictor for severity.

Country-specific analyses yielded distinct results. In Dutch patients, no almond components were associated with severity. A multivariable model including almond SPT sensitization and log-transformed Pru du y-conglutin, Pru p 3, and Bet v 1 levels showed moderate performance (AUC 0.74, 95% CI 0.61-0.87), again identifying Bet v 1 as a protective factor (OR 0.21, *p=0.034*) and almond SPT as predictor for severity (OR 8.37, *p=0.047*). Among Portuguese patients, a multivariable model including age, positive SPT to almond extract and log-transformed sIgE levels to Pru du 1 and Pru du 3 short, selected age and Pru du 1 as significant predictors. Older age and higher Pru du 1 levels were associated with milder symptoms (OR 0.92, *p=0.029*; OR 0.01, *p=0.023*, respectively). This model demonstrated strong severity discrimination (AUC 0.90; 95% CI 0.81-0.99).

Table 2. Univariate analyses on symptom severity (mild versus moderate-to-severe) in almond-allergic individuals

	Total population (N=122)				Netherlands (N=64)				Portugal (N=58)			
	oFASS-1 (N=54)	**oFASS 2-5 (N=68)**	***p***	**OR**	**oFASS-1 (N=28)**	**oFASS 2-5 (N+36)**	***p***	**OR**	**oFASS-1 (N=26)**	**oFASS 2-5 (N=32)**	***p***	**OR**
Demographics												
Age in years, mean (± SD)	39.13 ± 15.93	34.42 ± 13.32	0.080	0.98(0.95-1.00)	34.65 ± 12.11	34.70 ± 12.18	0.985	1.00(0.96-1.04)	44.30 ± 17.96	33.67 ± 14.67	**0.019**	0.96(0.93-0.99)
Male sex	11 (20.4)	26 (38.2)	0.054	2.42(1.06-5.51)	6 (21.4)	12 (33.3)	0.293	1.82(0.59-5.72)	5 (19.2)	14 (43.8)	**0.048**	3.27(80.98-10.84)
Clinical background												
Allergic rhinitis	49 (90.7)	57 (90.5)	0.961	0.97(0.28-3.37)	25 (89.3)	32 (88.9)	0.999	0.96(0.20-4.69)	24 (92.3)	25 (92.6)	0.969	1.04(0.14-8.00)
Prob grass pollen allergy	22 (56.4)	27 (58.7)	0.832	1.10(0.46-2.60)	6 (33.3)	12 (48.0)	0.336	1.85(0.53-6.48)	16 (76.2)	15 (71.4)	0.726	0.78(0.19-3.11)
Prob birch pollen allergy	34 (69.4)	28 (51.9)	0.069	0.48(0.21-1.07)	24 (88.9)	25 (75.8)	0.315	0.39(0.09-1.65)	10 (45.5)	3 (14.3)	**0.045**	0.20(0.04-0.88)
Allergic asthma	24 (46.2)	35 (55.6)	0.315	1.46(0.70-3.05)	10 (37.0)	20 (55.6)	0.145	2.12(0.77-5.90)	14 (56.0)	15 (55.6)	0.974	0.98(0.33-2.94)
Atopic dermatitis	24 (45.3)	37 (55.2)	0.279	1.49(0.72-3.07)	16 (59.3)	21 (60.0)	0.953	1.03(0.37-2.87)	8 (30.8)	16 (50.0)	0.139	2.25(0.76-6.65)
Sensitization												
SPT almond extract	30 (60.0)	33 (75.0)	0.123	2.00(0.82-4.85)	19 (73.1)	23 (88.5)	0.159	2.82(0.64-12.44)	11 (45.8)	10 (55.6)	0.533	1.48(0.43-5.05)
PTP almond extract	17 (70.8)	15 (71.4)	0.965	1.03(0.28-3.75)	0 (0.0)	1 (33.3)	-	-	17 (70.8)	14 (77.8)	0.612	1.44(0.35-5.95)
IgE almond extract	47 (87.0)	60 (88.2)	0.841	1.12(0.38-3.30)	26 (92.9)	31 (86.1)	0.454	0.48(0.08-2.66)	21 (80.8)	29 (90.6)	0.446	2.30(0.50-10.71)
IgE Pru du 1	45 (83.3)	46 (67.6)	**0.048**	0.42(0.17-0.99)	26 (92.9)	32 (88.9)	0.688	0.62(0.10-3.63)	19 (73.1)	14 (43.8)	**0.025**	0.29(0.09-0.87)

Table 2. Univariate analyses on symptom severity (mild versus moderate-to-severe) in almond-allergic individuals (*continued*)

	Total population (N=122)				Netherlands (N=64)				Portugal (N=58)			
	oFASS-1 (N=54)	**oFASS 2-5 (N=68)**	***p***	**OR**	**oFASS-1 (N=28)**	**oFASS 2-5 (N+36)**	***p***	**OR**	**oFASS-1 (N=26)**	**oFASS 2-5 (N=32)**	***p***	**OR**
IgE Pru du 3 (long)	24 (44.4)	32 (47.1)	0.773	1.11(0.54-2.28)	7 (25.0)	9 (25.0)	0.999	1.00(0.32-3.13)	17 (65.9)	23 (71.9)	0.595	1.35(0.44-4.13)
IgE Pru du 3 (short)	17 (31.5)	32 (47.1)	0.081	1.94(0.92-4.08)	2 (7.1)	9 (25.0)	0.095	4.33(0.85-21.99)	15 (57.7)	23 (71.9)	0.258	1.87(0.63-5.60)
IgE Pru du 2S albumin	2 (3.7)	1 (1.5)	0.583	0.39(0.03-4.40)	0 (0.0)	0 (0.0)	-	-	2 (7.7)	1 (3.1)	0.648	0.39(0.03-4.53)
IgE Pru du y-conglutin (7S)	4 (7.4)	12 (17.6)	0.117	2.67(0.97-7.33)	2 (7.1)	10 (27.8)	0.053	5.72(1.15-28.43)	2 (7.7)	2 (6.3)	0.999	1.27(0.32-5.08)
IgE Pru du 6 (11S)	1 (1.8)	4 (5.9)	0.385	2.27(0.57-8.90)	1 (3.6)	3 (8.3)	0.625	1.62(0.28-9.58)	0 (0.0)	1 (3.1)	-	-
IgE Bet v1	42 (77.8)	41 (60.3)	**0.040**	0.43 (0.19-0.97)	28 (100.0)	32 (88.9)	0.125	0.00 (0.00-)	14 (53.8)	9 (28.1)	**0.046**	0.34(0.11-0.99)
IgE Pru p3	23 (42.6)	41 (60.3)	0.052	2.05 (0.99-4.23)	5 (17.9)	16 (44.4)	**0.025**	3.68 (1.14-11.85)	18 (69.2)	25 (78.1)	0.442	1.59(0.49-5.17)
IgE levels (median, Q1-Q3)												
Almond extract	0.5(0.2-1.6)	0.5(0.2-1.5)	0.624	1.0(0.9-1.2)	0.6 (0.2-1.1)	0.4 (0.2-1.2)	0.240	1.1(0.9-1.4)	0.5(0.1-2.5)	0.6(0.2-2.0)	0.354	0.9(0.7-1.1)
Pru du 1	2.2(0.2-6.2)	0.6(0.0-3.3)	0.163	1.0(0.9-1.0)	4.5 (1.7-9.9)	3.3 (1.0-7.7)	0.802	1.0(1.0-1.0)	1.1(0.1-3.6)	0.1(0.0-0.5)	0.105	0.8(0.6-1.1)
Pru du 3 long	0.1(0.01-0.8)	0.1(0.0-1.3)	0.507	1.0(1.0-1.0)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.741	0.6(0.0-12.1)	0.9(0.0-5.4)	1.5(0.1-8.2)	0.513	1.0(1.0-1.0)
Pru du 3 short	0.1(0.0-0.5)	0.1(0.0-0.6)	0.537	1.0(1.0-1.0)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.260	23.5(0.1-5715.4)	0.5(0.0-5.8)	1.0(0.1-8.7)	0.537	1.0(1.0-1.0)

Table 2. Univariate analyses on symptom severity (mild versus moderate-to-severe) in almond-allergic individuals (*continued*)

	Total population (N=122)				Netherlands (N=64)				Portugal (N=58)			
	oFASS-1 (N=54)	**oFASS 2-5 (N=68)**	***p***	**OR**	**oFASS-1 (N=28)**	**oFASS 2-5 (N+36)**	***p***	**OR**	**oFASS-1 (N=26)**	**oFASS 2-5 (N=32)**	***p***	**OR**
Pru du 2S albumin	0.0(0.0-0.0)	0.0(0.0-0.0)	0.433	0.1(0.0-29.7)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.503	0.0(0.0-1595.0)	0.0(0.0-0.0)	0.0(0.0-0.0)	0.514	0.2(0.0-46.3)
Pru du y-conglutin (7S)	0.0(0.0-0.0)	0.0(0.0-0.1)	0.667	1.2(0.5-3.0)	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.418	1.7(0.5-5.9)	0.0(0.0-0.1)	0.0(0.0-0.1)	0.214	0.1(0.0-4.9)
Pru du 6 (11S)	0.0(0.0-0.0)	0.0(0.0-0.0)	0.357	7.0(0.1-441.6)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.426	6.5(0.1-649.3)	0.0(0.0-0.0)	0.0(0.0-0.1)	0.611	9.0(0.0-421.6)
Bet v 1	14.4(0.2-44.2)	1.9(0.0-17.9)	**0.042**	1.0(1.0-1.0)	35.9 (14.9-56.0)	15.0 (7.0-37.8)	0.142	1.0(1.0-1.0)	0.2(0.0-10.2)	0.0(0.0-0.1)	0.101	0.9(0.9-1.0)
Pru p 3	0.1(0.0-0.9)	0.2(0.0-2.3)	0.560	1.0(1.0-1.0)	0.0 (0.0-0.1)	0.0 (0.0-0.3)	0.130	3.6(0.5-24.4)	0.8(0.0-6.8)	2.2(0.1-11.8)	0.543	1.0(1.0-1.0)

DISCUSSION

This study is the first to compare almond component sensitization patterns and their clinical relevance between birch-endemic and Southern European regions. We observed clear geographical differences, with predominant Pru du 1 (PR-10) sensitization in the Netherlands and Pru du 3 (LTP) sensitization in Portugal, consistent with patterns described for other plant foods. Sensitization to Pru du 2S albumin was rare. Although almond extract and component sensitization overlapped substantially, a subset of extract-negative individuals showed isolated component sensitization, most often to Pru du 1, highlighting the potential added value of CRD when clinical suspicion persists despite negative extract-based tests.

The diagnostic performance of almond extract and individual components was limited and did not reach clinical utility, in line with earlier studies reporting poor diagnostic accuracy of almond sIgE.

Sensitization patterns across the Netherlands and Portugal

Our findings align with those reported for other allergenic plant foods.[17,31–33] Pru du 1 sensitization (PR-10 protein) dominated in NL, whereas Pru du 3 (both LTP isoforms) prevailed in PT.

In The Netherlands, strong correlations between Pru du 1 and Bet v 1 support birch pollen as the likely primary sensitizer in Northern Europe.[34,35] In contrast, Portuguese patients showed weaker Pru du 1-Bet v 1 correlations and stronger links with Cor a 1. This fits well with the low birch pollen exposure in Coimbra, making a role as primary sensitizer unlikely. Since hazel pollen is not very prevalent either, primary PR-10 sensitization probably originates from other trees, such as oak.[36,37]

The high prevalence of Pru du 3 sensitization in Portugal is consistent with peach-derived LTP sensitization (Pru p 3), a recognized pattern in Southern Europe, supported by strong correlations with Pru p 3.[9,32] These correlations were much weaker in the Netherlands, suggesting other foods or pollens contribute to LTP sensitization.[10]

Interestingly, sensitization to Pru du 2S albumin was virtually absent – only three borderline cases after SUMO correction (0.1-0.5 kU/L). This contrasts with other tree nuts and peanut, for which 2S albumins are major allergens associated with severe clinical reactivity.[31,38] Earlier suggestions of an almond 2S albumin from Poltronieri et al. were based on limited peptide homology, and later work by Che et al. proposed these peptides instead correspond to a vicilin-related hairpinin (Pru du 8) instead.[14,15] Our findings support the view that almond 2S albumin is unlikely to play a clinically relevant role. In contrast, sensitization to Pru du γ-conglutin (7S) and Pru du 6 (11S) was more frequent, particularly

in NL, suggesting that these storage proteins may be of greater relevance in Northern Europe, although numbers were small and samples from children (< 17 years) were not included.

Predictors for severity of almond allergy

Multivariable models showed that older age and PR-10 sensitization appeared to be associated with milder symptoms, especially in Portuguese patients. This aligns with reports linking PR-10 sensitization to milder phenotypes.[18,19] The age association should be interpreted cautiously, as age of symptom onset was not assessed and earlier onset is more often associated with more severe reactions.[31]

Overall, our findings suggest that PR-10 sensitization may contribute to a milder allergic phenotype in some populations. Larger, challenge-confirmed cohorts and longitudinal designs are needed to better understand the interplay between age, sensitization patterns, and clinical severity in almond allergy.

Strengths and limitations

A limitation of this study was the small number of Portuguese patients undergoing OFC. Although overall cohort sizes were adequate and the proportion of positive OFC outcomes was comparable between countries, the limited number of OFC-confirmed cases may have restricted the power of subgroup analyses. Additionally, multivariable severity models could not be limited to OFC-confirmed patients without significantly reducing statistical power.

Despite these constraints, this is the first study to compare almond component sensitization across two European regions, revealing patterns consistent with other plant-food allergens and providing new insights into geographic variation, sensitization origins, and diagnostic complexity.

CONCLUSION

This study demonstrates clear geographical differences in almond component sensitization, with Pru du 1 (PR-10) predominating in The Netherlands and Pru du 3 (LTP) in Portugal. Both for PR-10 and LTP, results further suggest different sources of primary sensitization in both countries. A subset of almond extract–negative patients were sensitized to individual components, most often to Pru du 1, and strong correlations were observed between Pru du 1 and Bet v 1, and between Pru du 3 isoforms and Pru p 3, with marked geographical variation in correlation strength. Convincing evidence for a role of Pru du 2S albumin as an almond allergen was not found. While almond component testing appears to currently lack the diagnostic accuracy to reduce the number of oral food challenges,

certain components – especially Pru du 1 – may provide complementary information to almond extract testing and assist in risk stratification of almond allergy, particularly for assessing reaction severity and phenotype.

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SUPPLEMENTAL

Supplemental 1. Almond component protein synthesis and ImmunoCAP conjugation

Component synthesis and purification

The six recombinant almond components were synthesized using the following process:

1. Gene cloning: DNA sequences encoding target almond proteins—based on the *Nonpareil* almond genome(27) – were commercially synthesized, cloned into one of two expression vectors, pE-SUMO3 for Pru du 2S albumin, Pru du γ-conglutin (vicilin-like 7S globulin), and Pru du 6 (11S globulin), or pET 16b for Pru du 1 and both Pru du 3s. Both were transfected into *Escherichia coli* (*E. coli*), strain BL21 (DE3) pLysS. The translated protein sequences are depicted in **Figure S1**. All constructs included a poly-histidine sequence (His-tag) for downstream purification. His-tag and, in case present the SUMO3-tag, were not removed. For control purposes, the SUMO tag was also produced as recombinant protein in a similar fashion;
2. Protein expression and extraction: recombinant proteins were expressed intracellularly in *E. coli*. Bacterial cells were lysed by sonication, and cellular debris was removed by centrifugation. All target proteins were mainly present in insoluble inclusion bodies;
3. Solubilization: inclusion bodies were solubilized using denaturing buffers (e.g., 2–8 M urea, pH 8). Solubilization efficiency and protein integrity were assessed by SDS-PAGE, and the optimal buffer conditions were selected for each protein;
4. Protein purification: solubilized proteins were purified via affinity chromatography using HisTrap HP columns (Cytiva, Marlborough, USA). Purity was assessed by SDS-PAGE;
5. Protein quantification: protein concentrations were determined using the BCA assay (Thermo Fisher Scientific).

Preparation for ImmunoCAPs

Purified recombinant proteins were biotinylated and immobilized onto streptavidin-coated ImmunoCAPs (Ro212, Thermo Fisher Scientific, Uppsala, Sweden) at 1.0 µg per test. This enabled quantification of allergen-specific IgE in patient sera and immunologic validation of the recombinant proteins.

Pru du 1 (PR-10)

GVFTYESEFTSEIPPPRLFKAFVLDADNLVPKIAPQAIKHSEILEGDGGPGTIKKITFGEGSQYGYVKHKIDSIDKENHSY-
SYTLTEGDALGDNLEKISYETKLVASPSGGSIIKSTSHYHTKGDVEIKEEHVKAGKEKASNLFKLIETYLKGHPDAYN

Pru du 3 (nsLTP - long form)

ITCGQVSSNLAPCIPYVRGGGAVPPACCNGIRNVNNLARTTPDRQAACNCLKQLSASVPGVNPNNAAALPGKC-
GVNIPYQISPSTNCANELDMGQVFSAADNIRESE

Pru du 3 (nsLTP - short form)

AVSCGQVVNNLTPCINYVANGGALNPSCCTGVRSLYSLAQTTADRQSICNCLKQAVNGIPYTNANAGLAAGLPGKC-
GVNIPYKISPSTDCKSIK

Pru du 2S (2S albumin)

AGGQCKRGGNKYIHGHLFHRERLLTGVCKRKECFALNVDVCCKELEDLDEPCRCPLLMEIVEAQRLFYNEIKFE-
EMPQKARKSPAFCHLALTGCPQSLLIYSL

Pru du 7S (7S albumin)

QDREQHCQKRCQDFKRPIIHSECIYQCRMWGPWSLPPAESSNHHKQQQQHEQEQQCKQRCQPQH-
GHRQQQQCQQECYEQIRQQREREEMQQMCQQSCEMQGSGGQQQQRQQCQRQCEQQVEQLKQCQL-
RCQMQGQGQQQEQCQRTCRQELEDQQRRQQQEQGPGGGDNKLEDETLNGEPRRRFEQCMQGCER-
QQQGQGEQEQCPRQCREQFDKEKRQYQQCKQSCEKSSQHDDEKRQCKQQCKQQISLQQGGGN-
QEEDDDALNGSGYGGAKERFEQCKLSCQSQQGQGQQEQCPKQCKQQFEHEKHQYKQCKRGCENQARD-
DVQKEQCKQQCTQQMSQQYGQQQEGSTGGGGLNQQQEEEGQMGSQGQSQSQSQSNNPFYFPSHRFQPRFQS-
NEGGLYVLERFTQRQSEVLRGIKNYRLAIFEARPNTFVLPHHCDAEAIYVVLSGQCTCTLLMQDRKESFNMEH-
GDVIRVPAGATTYLVNNNSDKTLRIAKLLQPVNNPGRFEEFFPAGSRNSESYFSVFSNDILESAFNTPREQLEHG-
FKQGQQQQGQGIVMRAPREQLQALSQAASSRRRGDRQSQGPFNLRQQRPVHSNNYGQFFEARPEEFNQLQDM-
DASVSCIEINQQAMMVPHFNSKATHLIMVVEGNGLVEMACPYLANQSQEIMGQQEQQGEQSGRYMKVTAQLSP-
GDVFVIPAGHPVALVAQNQNLRILGFGLYAQNNKRNFLAGQEDNIIMHMDREARQLAFGPEMEQIFSKQQQSYFVP-
TQQGRSRNQHLSSILEFAGVI

Pru du 6 (11 S albumin)

ARQSQLSPQNQCQLNQLQAREPDNRIQAEAGQIETWNFNQEDFQCAGVAASRITIQRNGLHLPSYS-
NAPQLIYIVQGRGVLGAVFSGCPETFEESQQSSQQGRQQEQEQERQQQQQGEQGRQQGQQEQQQER-
QGRQQGRQQQEEGRQQEQQQGQQGRPQQQQQFRQLDRHQKTRRIREGDVVAIPAGVAYWSYNDGDQEL-
VAVNLFHVSSDHNQLDQNPRKFYLAGNPENEFNQQGQSQPREQGEQGRPGQHQQPFGRPRQQEQQGNGN-
NVFSGFNTQLLAQALNVNEETARNLQGQNDNRNQIIRVRGNLDFVQPPRGRQEREHEERQQEQLQQERQQQG-
GQLMANGLEETFCSLRLKENIGNPERADIFSPRAGRISTLNTHNLPILRFLRLSAERGFFYRNGIYSPHWNVNAHSVVY-
VIRGNARVQVVNENGDAILDQEVQQGQLFIVPQNHGVIQQAGNQGFEYFAFKTEENAFINTLAGRTSFLRALPDEV-
LANAYQISREQARQLKYNRQETIALSSSQQRRAVV

Figure S1. Protein Sequences of Almond Components

Supplemental 2. Sensitization testing to homologous proteins from peanut and tree nuts

Within the PR-10 family, testing included rCor a 1 (hazelnut) in 61 patients (NL: 51; PT: 10) and rAra h 8 (peanut) in 39 (NL: 23; PT: 16).

LTP measurements included rCor a 8 (hazelnut) in 27 patients, rJug r 3 (walnut) in 19, and rAra h 9 (peanut) in 26 – all from Portugal only.

For SSPs, sIgE was measured against natural Cor a 9 (hazelnut, 11S globulin) in 62 patients (NL: 51; PT: 11), rCor a 14 (hazelnut, 2S albumin) in 55 from the Netherlands, rAra h 1 (peanut, vicilin-like 7S globulin) in 14 from Portugal, rAra h 2 (peanut, 2S albumin) in 58 (NL: 26; PT: 32), rAna o 3 (cashew, 2S albumin) in 11, and rJug r 1 (walnut, 2S albumin) in 10 from the Netherlands.

Correlations between components from each sub-family (using Spearman's rank correlation coefficient) are depicted in **Table S2.**

PR-10	**Bet v 1**			**Ara h 8**			**Cor a 1**		
	Overall	NL	PT	Overall	NL	PT	Overall	NL	PT
Pru du 1	0.84*	0.87*	0.60*	0.91*	0.87*	0.77*	0.92*	0.92*	0.91*
Total number	130	70	60	39	23	16	61	51	10
nsLTP	**Pru p 3**			**Ara h 9**	**Cor a 8**	**Jug r 3**			
	Overall	NL	PT	PT	PT	PT			
Pru du 3 long	0.77*	0.44*	0.93*	0.92*	0.92*	0.96*			
Pru du 3 short	0.82*	0.60*	0.92*	0.90*	0.94*	0.95*			
Total number	130	70	60	26	27	19			
2S albumins	**Ara h 2**			**Cor a 14**	**Jug r 1**	**Ana o 3**			
	Overall	NL	PT	NL	NL	NL			
Pru du 2S	-0.34*	-0.23	-0.30	0.01	0.35	0.47			
Total number	58	26	32	55	10	11			
7S globulins	**Ara h 1**								
	PT								
Pru du 7S	0.55*								
Total number	14								
11S globulins	**Cor a 9**								
	Overall	NL	PT						
Pru du 11S	0.29*	0.26*	0.47						
Total number	62	51	11						

Table S2. Heatmap with correlations between representatives of major protein groups and almond components (Spearman correlations)
*P-value < 0.05

Supplemental 3. Oral Food Challenges

OFCs were conducted as open challenges, with raw almond, and in accordance with local protocols based on the PRACTALL consensus guidelines.(29)

Three different dosing protocols were used across centers:

- The first protocol followed incremental doses of 0.001, 0.03, 0.1, 0.3, 1, 3, 10, 20, and 30 grams of almond.
- The second protocol used protein-based dosing, starting from 0.003 grams of almond protein and escalating through 0.01, 0.03, 0.1, 0.3, 1, and 3 grams.
- The third protocol involved a single 5-gram dose of almond.

Doses were administered every 20-30 minutes. Patients were monitored for at least two hours after the final dose or until symptom resolution.

An OFC was considered positive if subjective symptoms persisted for ≥ 45 minutes or if objective signs of an allergic reaction were observed. All challenge outcomes, including severity grading, were determined by an expert panel.

6

Ana o 3 is a better predictor of cashew nut allergy in adults than cashew nut extract

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Introduction

IgE against Ana o 3 is a better predictor of cashew nut allergy in children compared to IgE against cashew nut extract. Studies in adults are lacking. This study aimed to evaluate the diagnostic value of IgE against Ana o 3 versus cashew nut extract in adults with suspected cashew nut allergy, and their possible association with symptom severity.

Methods

Adult patients that underwent an oral food challenge (OFC) for suspected cashew nut allergy or presented with a history of anaphylaxis to cashew nut were included. The diagnostic value was assessed by receiver operating characteristic curve-analysis.

Results

In total, 18 allergic and 25 tolerant patients were included. IgE against Ana o 3 was superior in predicting cashew nut allergy with an area under the curve of 0.95 (95% CI 0.90-1.00) compared to 0.90 (95% CI 0.80-0.99) for cashew nut extract ($p_{DeLong's\ test} = 0.05$). Using cutoff levels for Ana o 3 with 100% positive predictive value (PPV) and 91% negative predictive value (NPV), 72% of patients were diagnosed compared to 49% when using the 100% PPV and 94% NPV cutoff levels for cashew nut extract. Although a limited association was observed visually, IgE against cashew nut extract and Ana o 3 did not statistically significantly distinguish between patients with mild/moderate and severe symptoms.

Conclusion

IgE against Ana o 3 is a good predictor of cashew nut allergy in adults, categorizing 72% of patients in our study population compared to 49% of patients when using IgE against cashew nut extract.

INTRODUCTION

Cashew nut allergy is a common tree nut allergy.[1,2] Allergic reactions to cashew nut vary from mild to severe.[3] Anaphylaxis occurs rather frequently in cashew nut allergic patients compared to other tree nut- and peanut-allergic patients.[3–5] Double-blind placebo controlled food challenge (DBPCFC) is the reference standard for diagnosis. However, this is costly, burdensome, and requires experienced personnel and dedicated hospital facilities.[6] In addition, some patients are excluded from DBPCFC (e.g. patients with previous severe anaphylactic reactions, uncontrolled asthma and pregnant women).[7]

Diagnostic tests that are less invasive and can be performed on every patient, include measurement of serum IgE levels against cashew nut extract, and more recently, cashew nut allergen components using component resolved diagnostics (CRD).[8,9] Thus far, characterized cashew nut components are the seed storage proteins Ana o 1 (vicilin-like 7S globulin), Ana o 2 (legumin-like 11S globulin) and Ana o 3 (2S albumin).[10] Of these components, sensitization to Ana o 3 is observed most frequently in cashew nut allergic children and is associated with triggering severe systemic reactions.[1112] Multiple studies in different countries demonstrated that IgE against Ana o 3 was superior in diagnosing cashew nut allergy in children compared to IgE against cashew nut extract (AUCs IgE against Ana o 3: 0.90 – 0.99; IgE against cashew nut extract: 0.78 - 0.89).[6,11,13–16] However, to our knowledge, the diagnostic value of IgE against Ana o 3 has not yet been studied in adults. In previous studies, it has been shown that the diagnostic value of components in the diagnosis of food allergy can be different between adults and children - for example in hazelnut, peanut and walnut allergy.[17–22]

Therefore, the aim of this study was to determine the diagnostic value of IgE against cashew nut extract and IgE against Ana o 3 in adults suspected of cashew nut allergy. In addition, the association between IgE levels against cashew nut extract and Ana o 3 and severity of cashew nut allergy was assessed.

METHODS

Subject selection

Adult patients (≥ 18 years) who visited the Department of Dermatology and Allergology of the University Medical Center Utrecht (UMCU), the Netherlands, with suspected cashew nut allergy who had previously undergone an open food challenge (OFC) or presented a clear history of anaphylaxis to cashew nut until June 2022 were included.

Patients were excluded from analyses if the time between IgE measurement and OFC was more than two years and/or if they had missing data on sIgE and insufficient serum available to determine sIgE results.

The study was approved by the local ethical committee (protocol number 13-272) and informed consent was signed by patients.

Data collection

Clinical data

Data on patient demographics (age, sex), allergic comorbidities (allergic rhinitis, asthma, atopic dermatitis), serum IgE levels against cashew nut extract and Ana o 3 and OFC results were retrospectively collected from patients' medical files. Anaphylaxis was diagnosed according to the Anaphylaxis guideline of the EAACI.[23]

IgE against cashew nut extract and Ana o 3

IgE levels against cashew nut extract and Ana o 3 were determined using the ImmunoCAP platform (ThermoFisher Scientific, Uppsala, Sweden). In patients without historic sIgE results, sIgE values were obtained using leftover serum stored in biobank. If possible, serum from the same timepoint was used to measure both IgE levels. Otherwise, available serum closest to the first measurement was used. Sensitization was defined as positive when sIgE levels were ≥ 0.35 kUa/L.

Open food challenge

OFCs were performed following the local protocol, based on the Practical Allergy (PRACTALL) guidelines.[24] The dosing scheme of the OFC was 0.01 gram - 0.03 gram - 0.1 gram – 0.3 gram – 1 gram – 3 grams - 10 grams and 20 grams of cashew nut. The OFC was considered positive when objective allergic reactions occurred or when subjective symptoms lasted at least 45 minutes. An expert panel decided on the outcome of the OFC. Symptom severity was graded using the validated oFASS-3 classification which divides allergic reactions into mild (oral cavity involved), moderate (skin, nose, eye, digestive and/or uterus system involved) and severe (larynx, bronchi, cardiovascular and/or nervous system involved).[25]

Data analysis and statistics

The characteristics of patients with cashew nut allergy to those without cashew nut allergy were described and compared statistically. P values ≤ 0.05 were considered statistically significant.

The correlation among IgE levels against cashew nut extract and Ana o 3 was calculated using Spearman's rho coefficients. The diagnostic value of IgE levels against cashew nut

extract and Ana o 3 was assessed by the area under the curve (AUC) of the receiver operating characteristic (ROC) analysis. DeLong's test was used to statistically compare the AUCs.[26] Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated for the clinically used cutoff point and the calculated optimal cutoff point as defined using the Youden index.[27] Cutoff points above which the PPV was 100% and below which the NPV was 100% were determined as well in our sample. If the cutoff point for the 100% NPV was below 0.10 kUa/L (the detection limit for accurately measuring IgE levels) the NPV corresponding to 0.10 kUa/L was used

The severity of cashew nut allergy was dichotomized as mild/moderate cashew nut allergy (i.e. oFASS-3 grade 1 and 2) versus severe cashew nut allergy (i.e. oFASS-3 grade 3). Differences in sIgE levels between tolerant patients, patients with mild/moderate and severe symptoms were tested using Kruskal-Wallis tests. AUC was used to further explore differences in IgE levels against cashew nut extract and Ana o 3 between patients with mild/moderate versus severe symptoms.

Analyses were performed using IBM SPSS Statistics (version 26.0.0.1) and RStudio (version 1.3.1093).

RESULTS

Clinical characteristics

A total of 52 patients underwent an OFC for suspected cashew nut allergy after which 9 patients were excluded from analyses due to insufficient serum volume availability and 2 due to exceeding the maximum amount of time between IgE measurement and OFC. Two patients were included based on a clear history of anaphylaxis to cashew nut. There was no significant difference in age, sex, atopic background and severity of allergic reaction during OFC between included and excluded patients. In total, 43 patients were included in this study.

Table 1 presents the patient characteristics. Of the 43 included patients, 18 (42%) were allergic and 25 (58%) were tolerant to cashew nut. Patients were significantly younger in the allergic group (25 years) than in the tolerant group (33 years) ($p = 0.015$).

Of the 18 allergic patients, 16 underwent an OFC and 2 had an anaphylactic history to cashew nut (**Table 2**). Of all allergic patients, 16 (89%) experienced objective symptoms and 2 (11%) only subjective symptoms. When classifying the allergic patients according to the oFASS-3 classification, none of the patients experienced a mild reaction, 12 (67%) had a moderate reaction and 6 (33%) a severe reaction.

Table 1. Patient characteristics

	Overall n = 43	Allergic n = 18	Tolerant n = 25	P-value
Sex, f*emale*	24 (56)	10 (56)	14 (56)	1.000
Mean age, *years (SD)*	29 (10)	25 (7)	33 (11)	**0.015**
History of atopic comorbidities				
Allergic rhinitis	35 (81)	16 (89)	19 (76)	0.434
Asthma	29 (67)	12 (67)	17 (68)	1.000
Atopic eczema	24 (56)	10 (56)	14 (56)	1.000
Median IgE against cashew nut, *kUa/L (IQR)*	0.30 (0.03, 2.18)	2.57 (0.48, 7.10)	0.05 (0.00, 0.31)	**<0.001**
Median IgE against Ana o 3, *kUa/L (IQR)*	0.08 (0.00, 1.80)	2.65 (0.28, 8.92)	0.01 (0.00, 0.07)	**<0.001**
Symptoms during OFC				
Objective	16 (37)	16 (89)	NA	
Subjective	2 (5)	2 (11)	NA	
None	25 (58)	NA	25 (100)	
Severity of allergic reaction during OFC				
oFASS score 1 (mild)	0 (0)	0 (0)	NA	
oFASS score 2 (moderate)	12 (28)	12 (67)	NA	
oFASS score 3 (severe)	6 (14)	6 (33)	NA	
None	25 (58)	NA	25 (100)	

Values are in n (%) unless otherwise indicated. T-test, Mann-Whitney U test or Chi-square test were used for statistics. P values in boldface indicate a significant difference between the allergic and tolerant group. Abbreviations: SD = standard deviation, *kUa/L* = kilo units of antibody per liter, *IQR* = interquartile range, *NA* = not applicable.

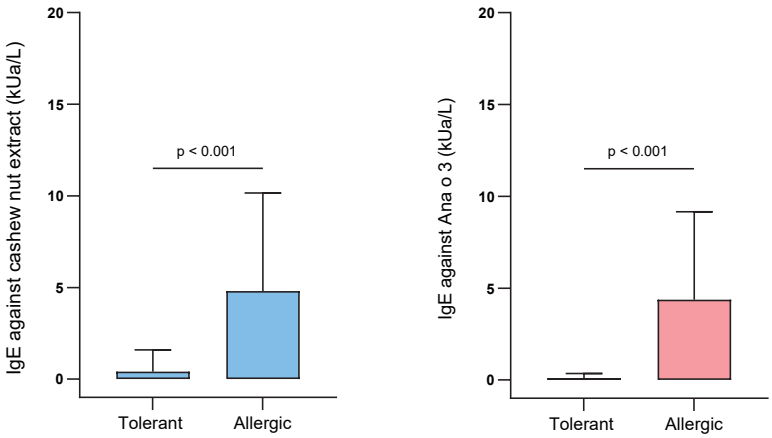


Figure 1. IgE levels against cashew nut extract and Ana o 3 in tolerant and allergic patients

Table 2. Detailed description of cashew nut allergic patients

No.	Sex	Age†	Symptoms	oFASS-3	Objective or subjective symptoms	OFC or anaphylaxis
1	Male	19	OAS, erythema, urticaria, dyspnea	Severe	Objective	OFC
2	Female	20	OAS, urticaria, conjunctivitis, nausea	Moderate	Objective	OFC
3	Female	23	OAS, urticaria, vomiting	Moderate	Objective	OFC
4	Male	29	OAS, urticaria	Moderate	Objective	OFC
5	Female	19	OAS, rhinitis, conjunctivitis, erythema, urticaria	Moderate	Objective	OFC
6	Male	19	OAS, swelling throat, nausea, hoarseness, dysphagia	Severe	Objective	OFC
7	Female	26	OAS, urticaria, nausea, vomiting	Moderate	Objective	OFC
8	Female	24	OAS, urticaria, coughing, dyspnea	Severe	Objective	OFC
9	Male	23	OAS, erythema, urticaria, abdominal pain, nausea	Moderate	Objective	OFC
10	Female	23	Nausea, diarrhea	Moderate	Objective	OFC
11	Male	27	OAS, nausea, abdominal pain, erythema, urticaria	Moderate	Objective	OFC
12	Female	34	OAS, nausea, vomiting, abdominal pain	Moderate	Objective	OFC
13	Female	22	Erythema, nausea, vomiting, diarrhea	Moderate	Objective	OFC
14	Male	45	OAS, erythema, itching skin, coughing	Severe	Objective	OFC
15	Male	18	OAS, objective swelling tongue and lips, vomiting, severe dyspnea, dizziness	Severe	Objective	Anaphylaxis
16	Male	30	OAS, severe dyspnea, wheezing, nausea, dizziness, disorientation	Severe	Objective	Anaphylaxis
17	Female	30	OAS, subjective swelling throat, abdominal pain, mild unsteady feeling without changes in blood pressure	Moderate	Subjective	OFC
18	Female	19	Subjective swelling mouth, abdominal pain	Moderate	Subjective	OFC

Abbreviations: OAS = oral allergy symptoms, O*FC* = *oral* food challenge

† Age at provocation in case a food challenge was performed, age at inclusion in case a patient was included based on a history of anaphylaxis

IgE against Ana o 3 is better than IgE against cashew nut extract at predicting cashew nut allergy

IgE levels against cashew nut extract and Ana o 3 were significantly higher in the allergic group (sIgE against cashew nut extract 2.57 kUa/L and Ana o 3 2.65 kUa/L) than in the tolerant group (sIgE against cashew nut 0.05 kUa/L and Ana o 3 0.01 kUa/L) (both $p < 0.001$) **Table 1**, **Figure 1**.

The correlation between IgE against cashew nut extract and Ana o 3 was strong (Spearman's rho: 0.92). IgE against Ana o 3 had a higher discriminative ability for predicting

cashew nut allergy in allergic versus tolerant patients (AUC of 0.95 [95% CI 0.90 – 1.00]) than IgE against cashew nut extract (AUC of 0.90 [95% CI: 0.80 – 0.99]) ($p_{DeLong's\ test}$ = 0.05) (**Figure 2**).

The diagnostic accuracy of IgE against cashew nut extract and IgE against Ana o 3 in allergic versus tolerant patients at different cutoff points is presented in **Table 3**. The lowest cutoff value above which PPV was 100% was 1.27 kUa/L for IgE against Ana o 3. Twenty-six percent (11/43) of the patients had an IgE level against Ana o 3 above this cutoff value. A value of <0.10 kUa/L for IgE against Ana o 3 resulted in a NPV of 91%, and identified 47% (20/43) of patients. When these values were combined, 72% of the patients could be diagnosed as cashew nut tolerant or allergic. When we combined the 100% PPV (cutoff value IgE > 5.82) and 90% NPV (cutoff value IgE <0.10) values for IgE against cashew nut extract, 49% (21/43) of the patients could be diagnosed as cashew nut tolerant or allergic.

Combining the calculated optimal cutoff point (both or either positive) for IgE against cashew nut extract and Ana o 3 did not further improve diagnostic accuracy.

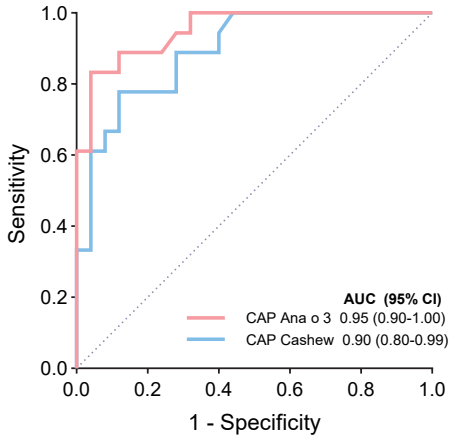


Figure 2. ROC-curves of IgE against cashew nut extract and Ana o 3 to predict cashew nut allergy. *Abbreviations: ROC, receiver operating characteristics; AUC,* area under the curve; *CI,* confidence interval.

Table 3. Measures of diagnostic accuracy for cashew nut allergy of IgE against cashew nut and against Ana o 3 applied to multiple cutoff points

	Cutoff point, *kUa/L*	**Sensitivity, *% (N/D)*[†]**	**Specificity, *% (N/D)*[‡]**	**PPV, *% (N/D)*[§]**	**NPV, *% (N/D)*[¶]**
Clinically used cutoff point					
Cashew nut	0.35	77.8 (14/18)	76.0 (19/25)	70.0 (14/20)	82.6 (19/23)
Ana o 3	0.35	72.2 (13/18)	96.0 (24/25)	92.9 (13/14)	82.8 (24/29)

Table 3. Measures of diagnostic accuracy for cashew nut allergy of IgE against cashew nut and against Ana o 3 applied to multiple cutoff points (*continued*)

	Cutoff point, *kUa/L*	Sensitivity, *% (N/D)*[†]	Specificity, *% (N/D)*[‡]	PPV, *% (N/D)*[§]	NPV, *% (N/D)*[¶]
Calculated optimal cutoff point					
Cashew nut	0.45	77.8 (14/18)	88.0 (22/25)	82.4 (14/17)	84.6 (22/26)
Ana o 3	0.23	83.3 (15/18)	96.0 (24/25)	93.8 (15/16)	88.9 (24/27)
Optimal PPV cutoff point					
Cashew nut	5.83	33.3 (6/18)	100 (25/25)	100 (6/6)	67.6 (25/37)
Ana o 3	1.28	61.1 (11/18)	100 (25/25)	100 (11/11)	78.1 (25/32)
Optimal NPV cutoff point					
Cashew nut	<0.10	94.0 (17/18)	60.0 (15/25)	63.0 (17/27)	93.8 (15/16)
Ana o 3	<0.10	88.9 (16/18)	80.0 (20/25)	76.2 (16/21)	90.9 (20/22)

Abbreviations: kUa/L = kilo units of antibody per liter, *N* = numerator, *D* = denominator, *FN* = false-negative results, *FP* = false-positive results, *NPV* = negative predictive value, *PPV* = positive predictive value, *TN* = true-negative results, *TP* = true-positive results.

[†] Numerator: TP, denominator: TP+FN; [‡] Numerator: TN, denominator: TN+FP; [§] Numerator: TP, denominator: TP+FP;

[¶] Numerator: TN, denominator: TN+FN

IgE levels of cashew nut extract and Ana o 3 are not significantly associated with severity

The median IgE levels of cashew nut extract and Ana o 3 were significantly higher in the mild/moderate and severe allergic patients compared to the tolerant patients (all p-values < 0.001) (**Figure 3**). Patients with a severe cashew nut allergy tended to have higher median IgE levels against both cashew nut extract and Ana o 3 than patients with mild/moderate cashew nut allergy, but this was not statistically significant (p = 0.43 vs p = 0.42, respectively). The AUCs of IgE levels against Ana o 3 and cashew nut extract in predicting severity were 0.69 [95% CI 0.43-0.96] and 0.68 [95% CI 0.41-0.94], respectively.

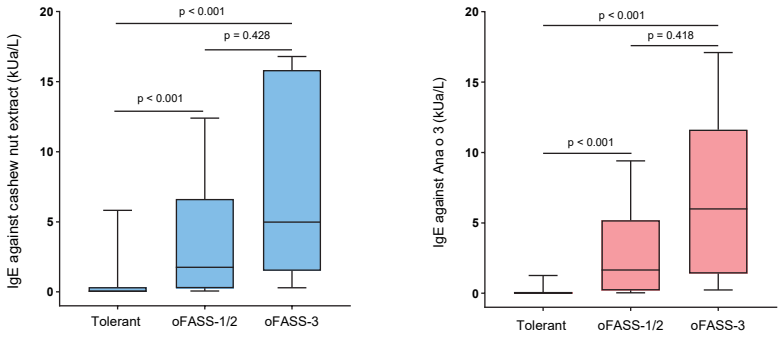


Figure 3. Level of IgE against cashew nut extract and Ana o 3 in tolerant patients, patients with mild/moderate symptoms (oFASS-1/2) and those with severe symptoms (oFASS-3). *Abbreviations: kUa/L* = kilo units of antibody per liter

DISCUSSION

Our study showed that IgE against Ana o 3 and cashew nut extract are both good predictors of cashew nut allergy in adults. However, IgE against Ana o 3 is superior to IgE against cashew nut extract. In our study, cashew nut allergy could be predicted accurately in 72% of adults using both the cutoff values for 100% PPV and for 91% NPV of Ana o 3. Although levels of IgE against cashew nut extract and against Ana o 3 tended to be higher in patients with severe reactions compared to patients with mild to moderation reaction, the results were not statistically significant.

IgE against Ana o 3 is superior to IgE against cashew nut extract in predicting cashew nut allergy

Our study is the first to investigate the diagnostic value of IgE against Ana o 3 versus cashew nut extract in adults. IgE against Ana o 3 and IgE against cashew nut extract were able to discriminate between cashew nut tolerant and allergic patients (AUC of 0.95 and AUC of 0.90, respectively). IgE against Ana o 3 was superior to IgE against cashew nut extract in the prediction of cashew nut allergy. These results are in line with previous studies in children. A Greek study by Savvatianos *et al*. investigated 63 cashew nut allergic children and 36 tolerant controls, demonstrating a near-optimal AUC of 0.98 for IgE against Ana o 3 compared to an AUC of 0.97 for IgE against cashew nut extract.[16] A Japanese study by Sato *et al*. included 26 allergic children and 69 tolerant children, demonstrating an AUC of 0.92 for IgE against Ana o 3 compared to an AUC of 0.79 for IgE against cashew nut extract.[15] Also a German[13], Australian[6], Finnish [14] and another Dutch [11] study confirmed these findings in children.

Seventy-two percent of patients can be accurately diagnosed using cutoff levels with 100% PPV and 91% NPV

When combining the cutoff value for 100% PPV (>1.27 kUa/L) and 92% NPV (<0.10 kUa/L) for IgE against Ana o 3, a total of 72% of patients were diagnosed as either cashew nut allergic or tolerant. Only for patients with an IgE level against Ana o 3 between 0.10 and 1.27 kUa/L (28%) would an OFC be needed. Measuring IgE against cashew nut extract had no added value when Ana o 3 had already been measured.

For children, as far as known, only one study showed cutoff levels with 100% PPV and 100% NPV for IgE against Ana o 3 and cashew nut extract with comparable results; an OFC was needed in 19% versus 66% respectively.[6] As PPV and NPV of diagnostic tools are dependent on the prevalence of cashew nut allergy in the population examined and our patient sample was relatively small, generalizability of these cutoff values to other populations is unclear, stressing the importance of validation in other datasets.

IgE against Ana o 3 and cashew nut extract do not predict severity of cashew nut allergy

In our study, the median IgE values against cashew nut extract and Ana o 3 were significantly higher in the allergic patients with mild/moderate and severe symptoms compared to the cashew nut tolerant patients. Although levels of IgE against cashew nut extract and against Ana o 3 tended to be higher in patients with severe reactions compared to patients with mild/moderate reactions, this was not statistically significant. This might be due to the small number of cashew nut allergic patients (12 patients with moderate vs 6 patients with severe allergic reactions). An earlier Dutch study (n=173) obtained comparable results in children and found a significant difference in IgE levels against cashew nut extract and against Ana o 3 between the tolerant and cashew nut allergic group ($p<0.001$), and no significant difference in both IgE levels between patients with mild symptoms and anaphylaxis ($p=0.916$).[11] A Finnish study (n=106) identified a significant difference in IgE level between a tolerant group and cashew nut allergic group ($p<0.001$) but did not mention the p value between the mild versus moderate-to-severe reaction during OFC.[14]

Strengths and limitations

Our study has some limitations. Firstly, food challenges were performed open and not double blind, placebo-controlled. Secondly, our group of patients was relatively small, including 18 cashew nut allergic patients.

Nonetheless, this is the first study to evaluate and compare the diagnostic value of IgE against Ana o 3 and IgE against cashew nut extract in adults. All patients underwent OFC or had a history of anaphylaxis to cashew nut and were therefore excluded from undergoing food challenges.

CONCLUSION

In conclusion, this study showed that IgE against Ana o 3 is superior to IgE against cashew nut extract in predicting cashew nut allergy. Although an association with symptom severity was observed, IgE against Ana o 3 and against cashew nut extract were not able to predict severity of cashew nut allergy. Using cutoff levels for IgE against Ana o 3 with 100% PPV and 91% NPV could accurately diagnose 72% of patients. Using these cutoff levels, after validation, in clinical practice, would have the potential to strongly reduce the number for food challenges needed to diagnose cashew nut allergy.

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7

Continuously monitoring may predict an allergic reaction during an oral food challenge: an exploratory study

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Background

Very few studies have used continuous monitoring to predict allergic reactions during oral food challenge (OFC).

Objective

This pilot study explores whether measurements performed by CheckPoint Cardio Wireless System (sensor) during an OFC could predict an allergic reaction of patients before objective symptoms occur.

Methods

During OFC, adult patients were continuously monitored for respiratory rate (RR), oxygen saturation (SpO2), temperature (T), heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and QT interval (QT). Changes in parameters during the last 10 minutes prior to different objective allergic reactions (OAR) or end of OFC were compared between objectively allergic patients and no/subjectively allergic patients, respectively, using linear mixed effect modelling. Changes in parameters over time were plotted relative to the occurrence of objective symptoms. A random forest model was used to predict discontinuation of an OFC due to objective symptoms.

Results

In total, 74 patients were included, of whom 14 were objectively allergic, and 60 not/subjectively allergic. HR, DBP, and SBP significantly increased before gastrointestinal and pulmonary symptoms and decreased before cardiovascular symptoms. RR and SpO2 significantly increased before gastrointestinal symptoms, decreased before pulmonary symptoms, and SpO2 decreased before cardiovascular symptoms compensated by an increase in RR. It may be possible to predict an OAR leading to discontinuation of an OFC with promising accuracy of about 60-70%, but the model was yet unstable, likely due to limited patients.

Conclusion

Continuous monitoring may be useful in early detection of a food allergic reaction, leading to better and earlier observation and interpretation of symptoms.

INTRODUCTION

The gold standard for diagnosing a food allergy (FA) is an oral food challenge (OFC). The severity of a reaction that could occur during OFC can range from mild to severe and even life-threatening.[1–3] An OFC results in anaphylaxis in 2.4% of the cases.[4] Therefore, an OFC is often perceived as a stressful experience for both patient and healthcare professional.

Physiological parameters change during an allergic reaction. As far as known, little is known about these changes during an OFC. What is known, is that during a DBPCFC, acute objective reactions were associated with significant changes in stroke volume, heart rate, and peripheral blood flow, regardless of the type of symptoms.[5] Furthermore, during OFCs with egg, a significantly higher mean nasal temperature was measured in patients with a positive challenge compared with those with a negative challenge.[6]

Most patient complications do not occur suddenly, but develop over time.[7–10] It is therefore expected that physiological parameters changes preceding an allergic reaction. Early detection of alterations in physiological parameters during OFCs provides opportunities for early recognition, intervention and prevention of a (severe) reaction. This might be possible by using continuous monitoring of these physiological parameters.[11] Continuous monitoring can also help nursing staff save time to focus on clinical care instead of manually measuring parameters.[11] Furthermore, manual measurement and documentation of physiological parameters is often incomplete[12,13] and potentially less accurate as these per minute are often only assessed for 15 or 30 seconds[11]. Many of the shortcomings of intermittent manual physiological parameter measurements can be overcome by automated continuous monitoring, which is also observer independent.[11]

The technology of wearable wireless sensors for monitoring physiological parameters is advancing rapidly.[14] As physiological parameters change during an allergic reaction, sensors may have potential for (early) detection of an allergic reaction.[5]

The Checkpoint Cardio Wireless System (CCWS) is a small wearable sensor worn on the patient's chest and used for this pilot study. It is able to measure 28 physiological parameters every 20 seconds of which 7 were selected for this pilot study based on data quality and expert opinion. The aim of this pilot study was to investigate whether measurements taken by the CCWS during an OFC can predict an allergic reaction of a patient before the onset of objective symptoms.

METHODS

Study design

This prospective exploratory study was carried out from September 2021 till November 2022 at the Dermatology and Allergology Department of the University Medical Centre Utrecht (UMCU), The Netherlands. Adult patients with a suspected food allergy, who underwent an oral food challenge (OFC) were eligible to participate. Written informed consent was obtained prior to inclusion. Excluded from this study were breastfeeding women, patients with a known allergic reaction to ECG-stickers and/or patients with a pacemaker or Implanted Cardioverter Defibrillator (ICD).

The study was approved by The Medical Research Ethics Committee (MREC)(number 21-422) and informed consent was obtained from each participating patient.

Data collection

Oral food challenge

OFCs were performed as part of routine care according to a protocol based on the PRACTALL consensus report.[15] The OFC was performed open, single blind (SBPCFC) or double-blind placebo-controlled (DBPCFC). Both SBPCFC and DBPCFC existed of a placebo and verum day and an open challenge with one or more open portions of the unprocessed food followed directly after day two. Every 20-30 minutes a new portion was administered.

During a challenge, a specialized nurse documented the time that the food was administered, the symptoms that occurred, and the time that these symptoms occurred and resolved. When necessary, symptoms were treated with antihistamine, dexamethasone and/or epinephrine. All patients were observed for at least 2 hours after administering the last portion or after a reaction was ceased. Stop criteria were based on PRACTALL guidelines.[16] An expert panel decided on the outcome of the challenge. Symptom severity was graded from 0 to 4 according to the modified Mueller classification.[17,18]

Checkpoint Cardio wireless system

Before the start of the challenge the Checkpoint Cardio wireless system (CCWS) was attached to the patient. This study used the Multiple Physiological Parameter Ambulatory Telemonitoring System Model CPC12S which is a non-invasive, wireless sensor with five ECG electrodes (3M), a temperature sensor located in the axilla and an ear sensor located in the earlobe. For this pilot study, every 20 seconds the CCWS measured the following 7 physiological parameters: 1) respiratory rate (RR), 2) oxygen saturation (SpO2), 3) temperature (T), 4) heart rate (HR), 5) systolic blood pressure (SBP), 6) diastolic blood pressure

(DBP), and 7) QT interval (time between ventricular de- and repolarization and therefore inversely proportional to HR, [QT]).

A recent study measuring the accuracy of this sensor showed that the sensor accurately measured HR, slightly overestimated RR and SpO2, underestimated T and had a low accuracy for BP. QT was not included in this study.[19] Although the absolute values were not always as good as the reference values, the changes over time were quite comparable.

Data analysis

No formal sample size analysis was performed for this exploratory pilot study, but we aimed to include at least ten patients with an objective allergic reaction.

Data on patient demographics, BMI and symptom severity were described using means with standard deviation (SD) or medians with interquartile ranges (Q1, Q3) for respectively normally or non-normally distributed continuous variables, and frequencies and proportions for categorical variables. Differences between objective allergic, subjective allergic, and tolerant patients were tested using ANOVAs, Kruskal-Wallis tests, or Fisher's exact tests, respectively.

After OFC, the included patients were divided into objectively allergic and patients that were subjectively allergic or not allergic by the expert panel. Patients with an inconclusive challenge and those where the sensor did not provide any output data were excluded from analysis. We defined four types of objective allergic symptom: 1) symptoms relating to skin or mucous membrane, including swelling of lips and/or eyelids, swelling of tong/or throat, rhinitis, sneezing, allergic conjunctivitis, rash, erythema and/or urticaria; 2) gastrointestinal symptoms, including vomiting and/or diarrhea; 3) pulmonary symptoms, including hoarseness, coughing, wheezing and/or objective dyspnea with or without drop of peak flow; and 4) cardiovascular symptoms, including collapse, change in heart rate and/or blood pressure. An "*objective allergic reaction (OAR)*" was defined as the first occurrence of each of the four types of objective symptoms. Therefore, a patient could have a maximum of four OAR, i.e. skin/mucous membrane OAR, gastrointestinal OAR, pulmonary OAR, and cardiovascular AOR. "*First objective allergic reaction (FOAR)*" was defined as the occurrence of the first OAR, irrespective of the type of symptoms, so a patient could only have one first OAR.

Three types of analysis were performed to explore the data.

Change in individual parameters prior to objective event

As an initial exploration whether any of the seven parameters changed over time prior to the OAR, linear mixed effects modelling[20] was used, with separate analyses for each parameter and type of OAR. For this analysis, the 10-minute period up to the OAR (for ob-

jective allergic patients) or the last 10 minutes of the OFC (for subjective allergic/tolerant patients) were used. This time period was used because this was a pilot study and it gives the healthcare provider enough time to prepare rescue medication. The parameters were used as dependent variables. The occurrence of an OAR (yes/no), time in minutes, and the interaction term between time and OAR were used as independent variables.

Visual exploration of parameters over time

As multiple time windows may be relevant between changes in parameters over time and the OAR, we further explored the data visually. For this analysis, we used the FOAR (instead of the earlier analysis where we used the OAR) to prevent influence of parameters by a previous OAR. To summarize changes in parameters over time in a patient, per scaled parameter we calculated the standard deviation (SD) over a certain time window starting from the first observation, and then from each following observation (rolling SD). Time windows of 5, 10, 15 and 20 minutes were used to calculate this rolling SD. The mean times seven of the rolling standard deviations of the seven variables per time window (sum) was also calculated as a measure of overall change in parameters. The rolling SDs (per parameter and overall) were plotted over time per objectively allergic patient (separate for aggregation windows of 5, 10, 15 and 20 minutes) up to the FOAR with an overview of the given doses during OFC and compared to similar plots for tolerant patients to explore optimal time-windows and aggregation periods for predicting an objective event.

Prediction of objective event leading to discontinuation of OFC

Ideally, we would like to be able to predict the OAR that leads to the discontinuation of an OFC (i.e. discontinuation OAR) before the dose is given that led to the discontinuation. This might enables the prevention of this reaction or may not be as severe. Therefore, we performed a random forest analysis with discontinuation OAR yes/no as outcome and aggregates of parameter values over time as predictors. A period was defined as the time between two doses. The period after the dose that caused the discontinuation OAR/the final dose was defined as T0, the period before T-1 (time between the last and next to last dose), and so on. Each of the seven parameters, HR, RR, SpO2, T, DBP, SBP, and QT, were summarized for each period in different ways; mean, range (max-min), and change for mean and range (the difference between the current period and the period before the current period). As there was much more data available for patients without a discontinuation OAR (i.e. subjectively allergic/tolerant patients) than for patients with, and in order to have a reasonable sized set of data for the patients with a discontinuation OAR, the last four periods before the dose that caused the discontinuation were used for patients with a discontinuation OAR (T-4 up to and including T-1), and the last two periods were used for patients without a discontinuation OAR (T-2 up to and including T-1).

To further accommodate for the difference in population size between the groups with and without a discontinuation OAR, the models were run ten times using cross-validation. The sample size per run was set to 90% of the available datapoints for the patients with a discontinuation OAR, using the last 10% to validate. For the patients without a discontinuation OAR, a sample of the same size was drawn for each repetition, so each of the ten repetitions contained the same number of periods for the patients with and without a discontinuation OAR to make sure both groups were weighed equally in creating the random forest model.

Analyses were performed using IBM SPSS Statistics version 27 (2020, IBM, Armonk, NY, United State of America), and R version 4.3.3 (2022, R Foundation for Statistical Computing, Vienna, Austria) using the randomForest package (version 4.7-1.1, Liaw A, Wiener M (2002). "Classification and Regression by random Forest." R News, 2(3), 18-22) for the random forest model.

RESULTS

Study population

The CCWS was used during a total of 85 challenges. Patients with an inconclusive challenge (n=6), and patients where CCWS technical errors occurred (n=5) were excluded. After exclusion, 74 challenges were analyzed of which 14 were classified as objectively allergic, 16 as subjectively allergic and 44 as tolerant (**Table 1)**. The mean age of the total group was 37.5 years (SD 13.4) and 69.9% were female. Among the objectively allergic patients, 28 OAR occurred; 13 skin/mucous membrane OAR, 9 pulmonary OAR, 4 gastrointestinal OAR and 2 cardiovascular OAR. The Mueller score of the objectively allergic patients ranged between Mueller 1 to 4, with Mueller 3 most frequent scored (50%).

Table 1. Characteristics of study population

Baseline characteristics, N (%)	Total N = 74	Objectively allergic N = 14	Subjectively allergic N = 16	Tolerant N= 44	P-value
Age (mean, SD)	37.5 (13.4)	39.0 (14.1)	32.6 (12.1)	38.9 (13.4)	0.245
Female	51 (69.9)	10 (71.4)	11 (68.8)	30 (69.8)	0.987
BMI (median, Q2-Q4)	25.4 (22.2-29.3)	26.0 (21.2-28.3)	26.9 (25.2-32.7)	25.1 (22.1-29.2)	0.591
Provocation type					0.403
Open provocation	59 (80.8)	12 (85.7)	11 (68.8)	36 (83.7)	
DBPCFC	13 (17.8)	2 (14.3)	4 (25.0)	7 (16.3)	
SBPCFC	1 (1.4)	0 (0.0)	1 (6.3)	0 (0.0)	
Objective allergic reaction					NA
Skin/mucous membrane	13 (17.8)	13 (92.9)	NA	NA	

Table 1. Characteristics of study population (*continued*)

Baseline characteristics, N (%)	Total N = 74	Objectively allergic N = 14	Subjectively allergic N = 16	Tolerant N= 44	P-value
Gastrointestinal	4 (5.5)	4 (28.6)	NA	NA	
Pulmonary	9 (12.3)	9 (64.3)	NA	NA	
Cardiovascular	2 (2.7)	2 (14.3)	NA	NA	
Mueller classification					**< 0.001**
Mueller 0	9 (12.3)	0 (0.0)	9 (56.3)	NA	
Mueller 1	5 (6.8)	4 (28.6)	1 (6.3)	NA	
Mueller 2	6 (8.2)	1 (7.1)	5 (31.3)	NA	
Mueller 3	8 (11.0)	7 (50.0)	1 (6.3)	NA	
Mueller 4	2 (2.7)	2 (14.3)	0 (0.0)	NA	

All measurements are in n (%), unless otherwise specified

Abbreviations: NA= not applicable, BMI= Body Mass Index, DBPCFC= double-blind, placebo-controlled, food challenge, SBPCFC= double-blind, placebo-controlled, food challenge, SD = standard deviation, Q2-Q4 = quartile 2 – quartile 4 of interquartile range

Course of parameters measured by CCWS during a challenge

During OFC, variable patterns of the seven parameters over time were seen. **Figure 1** shows an example of the course of these parameters over time during OFC in an individual patient with all four types of OAR. In this patient, there were slight fluctuations in the parameters around each dose of the OFC, which increased after the last dose. The changes of the parameters over time before the different OAR of this patient were quite comparable with all the patients with an OAR and will be described below.

The change of the parameters over time in the patients with an OAR compared to those without in the ten minutes before an OAR or end of OFC, respectively, is shown in **Table 2.** All parameters showed a statistically significant association in at least two types of OAR and only SBP changed before all four types of OAR.

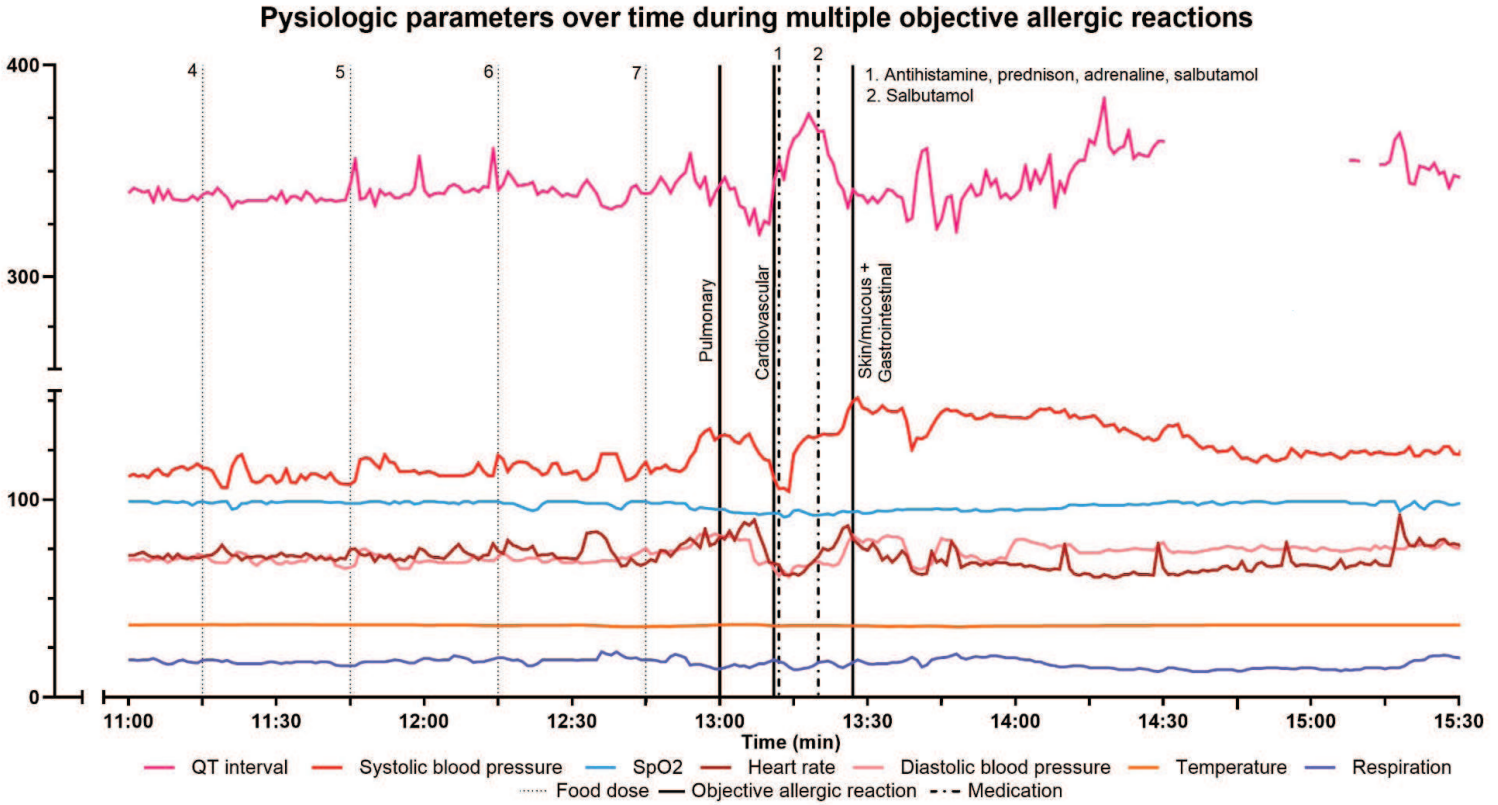


Figure 1. Overview of the seven parameters over time during an oral food challenge with all four objective events
The y-axis represents the value of the parameters and the X-axis the time per 4 minutes. The time starts two hours after the first portion

Depending on the type of OAR, parameters increased or decreased, with a distinction being made between cardiovascular parameters (i.e. HR, SBP, DBP, QT) and pulmonary parameters (i.e. RR and SpO2) **(Table 2**). There was an increase in HR and SBP and, as expected, a decrease in QT before any or all skin/mucosal OAR, gastrointestinal OAR and pulmonary OAR. Only the DPB had a different direction for skin/mucous membrane OAR (decrease) than for gastrointestinal OAR (increase). HR, SBP and DPB all decreased before the cardiovascular OAR. Regarding the pulmonary parameters, both RR and SpO2 increased before the gastrointestinal OAR, decreased before the pulmonary AOR, and there was a decrease in SpO2 compensated by an increase in RR before the cardiovascular AOR. The temperature only increased before the skin/mucous membrane OAR and pulmonary OAR. This shows that different types of OAR might be associated with different changes in parameters.

Table 2. Differences in changes over time of parameters in patients with a specific objective allergic reaction compared to those without in the last ten minutes before a reaction or end of OFC

Parameter	Skin/mucous membrane	Gastrointestinal	Pulmonary	Cardiovascular
Respiratory rate		↑	↓	↑
SpO2		↑	↓	↓
Temperature	↑		↑	
Heart rate	↑	↑		↓
Systolic blood pressure	↑	↑	↑	↓
Diastolic blood pressure	↓	↑		↓
QT interval	↓	↓	↓	

Direction: Increase (↑) or decrease (↓) of parameter over time in patients with an OAR (10 minutes preceding OAR) compared to those without (10 minutes preceding end of challenge)

Color: ↑↓ P-value of ≤ 0.05; ↑↓ P-value of ≤ 0.01; ↑↓ P-value of ≤ 0.001
Empty cell means there is no significant difference
To correct the analysis for the difference in the time of day when the measurements were taken, time-to-OAR (as well as its quadratic form to allow for non-linear associations) was added as covariate. A parameter was considered possibly predictive when a significant difference (p value ≤ 0.05) was seen in course over time of the parameter between patients with an OAR and patients without an OAR.

Unclear difference between changes of the parameters over time between allergic and tolerant patients

As we wanted to investigate whether changes of the parameters over time might also be relevant in the time before the last ten minutes, we further explored the data visually from the first dose until the *first objective allergic reaction (FOAR)* (objective allergic patients) or the end of the OFC (tolerant patients). In total, there were five skin/mucous membrane FOAR, one gastrointestinal FOAR, four pulmonary FOAR, three skin/mucous membrane FOAR combined with gastrointestinal FOAR and one skin/mucous membrane FOAR combined with a pulmonary FOAR. There was no cardiovascular FOAR. **Figure 2** shows examples of the changes in the rolling SD of the seven parameters over time separately and together (sum) for a patient with a pulmonary FOAR and a patient with a skin mucous membrane FOAR (**2A**) and two patients without a FOAR (**2B**). It seems that for both patients with and without a FOAR, the sum of the rolling SD fluctuated around each dose, mostly due to changes in RR, HR, and QT. Most patients with a FOAR showed an increase in the sum of the rolling SD during the consecutive doses up to the last dose before the FOAR, after which this increase continued or decreased until the FOAR. Most patients without a FOAR did not show an increase in the sum of the rolling SD during the consecutive doses up to the last dose, but did show an increase at the end of OFC, just before the sensor was removed. This was mostly due to an increase in all variables except SpO2 and T. However, there were large inter- and intra-person differences. Overall, it might be that the difference in parameters over time between patients with and without a FAOR is greater in the periods between the last consecutive doses than in the last ten minutes before an AOR or end of OFC.

Prediction of an objective allergic reaction before the final dose that leads to discontinuation of the OFC

As there might be differences in the parameters over time before the last dose in patients with a FOAR compared to those without, we investigated whether measurements in the periods between subsequent doses before an objective reaction that leads to discontinuation of an OFC could predict the occurrence of that discontinuation OAR.

For the analysis, we aggregated the results for six of the seven variables. As SpO2 measurements were very sparse, SpO2 was not included in these analyses.

For predicting a discontinuation OAR, we ended up with 204 periods in the complete dataset, of which 41 belonged to subjects with an OAR. Over the different cross-validation sets, the sensitivity (i.e., the percentage of discontinuation OAR that were correctly predicted to be a reaction) and specificity (i.e., the percentage of non-events that were correctly predicted to not be a reaction) showed a high variability, with sensitivity ranging from 0.2 to 1 and specificity ranging from 0.4 to 1, and as a result accuracy ranging from 0.3 to 0.9. The variable importance showed similar variability,

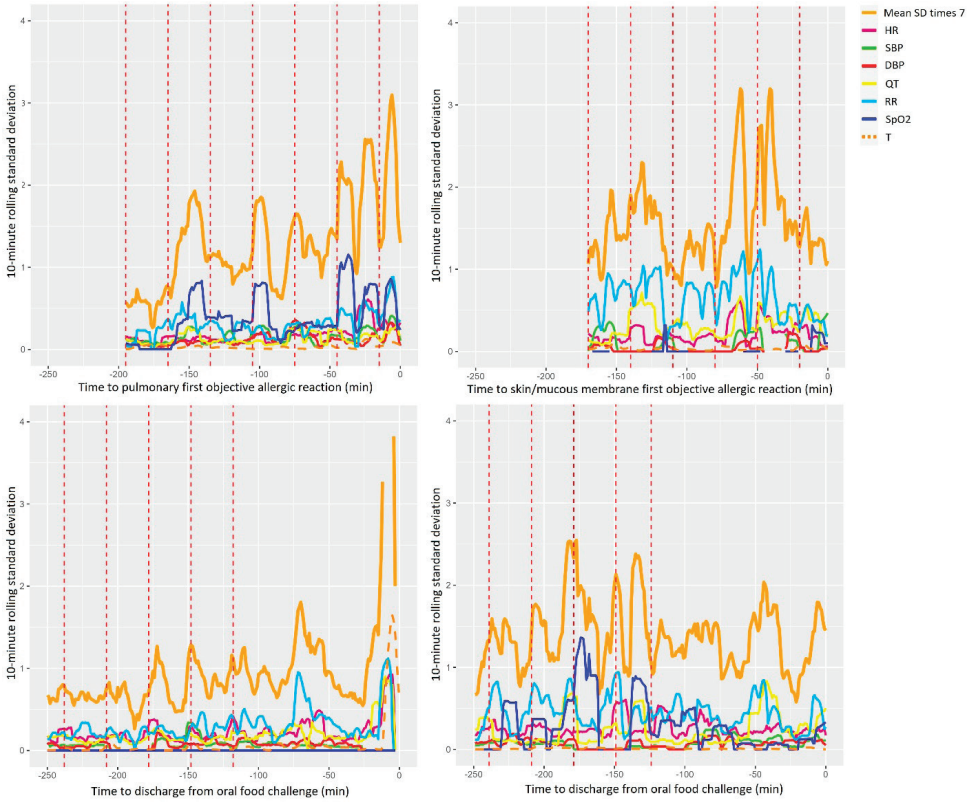


Figure 2. The variation per parameter over consecutive 10 minute periods (expressed as rolling standard deviation) as well as the mean variation of all parameters for a patients with a FOAR (**2A**) and without (**2B**)

T0 = time to FAOR (objective allergic patient) or end of OFC (tolerant patient) and '**-**…' the minutes before the FAOR/end of OFC. To optimally use available data and account for missing parameters in period due to <2 measurements, the mean the SDs was calculated as the mean of available parameters multiplied by 7 (sum)

Abbreviations: FAOR = first allergic objective reaction, OFC = oral food challenge, HR = heart rate, SBP = systolic blood pressure, DBP = diastolic blood pressure, QT = QT-interval, RR = respiration rate, SpO2 = oxygen saturation, T = temperature

with most variables being sometimes among the most important, but in other cases among the least important. As such, the results indicate that the data is too sparse and the variability too large to get a robust result.

Based on the cross validation results, three models were fitted to the full dataset: one using the mean and range of the parameters, one using the change between two time periods, and one using the mean values, range and change (i.e. full model). For the full model and the model using the mean and range, the specificity and accuracy was 0.9. The sensitivity was 1 and 0.9, respectively. For the model using the change values, the

sensitivity, specificity and accuracy were above 0.8. It is important to note that these models predicted the same data as they had used (extensively) in their training so these results must be approached with the utmost caution.

When recreating the full model using only the time between the fourth to last dose to next to last dose (T-4 to T-2) of the objectively allergic patients and the subjectively-/non-allergic patients to train the model, and using the time between the next to last dose and last dose (T-1) to validate the model, a sensitivity of 0.7, and specificity and accuracy of 0.6 was found. This indicates that there was a 60% accuracy in prediction whether or not patients would experience a discontinuation OAR based on the current dose. This is better than chance, but more research is needed to confirm and/or improve this approach.

DISCUSSION

To the best of our knowledge, this is one of the first studies that has evaluated the potential of a wireless sensor to predict an allergic reaction before the onset of objective symptoms during an OFC. This pilot study showed that the sensitivity, specificity and accuracy of the validated models to predict a discontinuation OAR were about 0.6 to 0.7, but the results were unstable given the small dataset. This indicates that it might be possible to predict an objective allergic reaction leading to discontinuation of an OFC. We also showed that there was an increase in HR, DBP and SBP, and a decrease in QT before any or all skin/mucosal OAR, gastrointestinal OAR and pulmonary OAR. Only the DPB decreased before a skin/mucous membrane OAR. HR, SBP and DPB all decreased before the cardiovascular OAR. Regarding the pulmonary parameters, both RR and SpO2 increased before the gastrointestinal OAR, decreased before the pulmonary event, and there was a decrease in SpO2 compensated by an increase in RR before the cardiovascular AOR.

Physiological parameters might predict an objective allergic reaction

The results of the exploratory random forest models suggest that it might be possible to predict an objective allergic reaction leading to discontinuation of an OFC during a period before the dose that caused the objective allergic reaction. However, the current dataset is too small to be certain, indicated by the large variation when using a cross-validation approach. This was also the reason why each period in this analyses was considered as an independent measure. Including a hierarchical approach would complicate the analyses to a point where the data would be insufficient for even exploratory analyses.

Other studies also showed promising results. Ruiz-Garcia et al. investigated changes in cardiovascular parameters over time up to multiple objective symptoms (skin, gastrointestinal, and respiratory) during OFCs with peanut.[5] They showed that objective allergic reactions were associated with significant changes in stroke volume, heart rate, and

peripheral blood flow that were already visible before the last given dose that caused an objective allergic reaction. Clarke et al showed that there was a significantly higher mean nasal temperature in patients with a positive OFC with egg and that the highest nasal temperature increase during challenge was already measured 20 minutes after the first dose.[6]

Different types of objective allergic symptoms may be associated with different changes in physiological parameters

Different changes in physiological parameters were observed before the different types of objective allergic reactions (i.e., skin/mucous membrane symptoms, gastrointestinal symptoms, pulmonary symptoms, and cardiovascular symptoms), which might be because of a different pathophysiology. Below, we explore this and distinguish between changes in vascular and pulmonary parameters.

During an IgE-mediated allergic reaction, mast cell degranulation leads to the release of histamine and pro-inflammatory cytokines, resulting in i.e. increased vascular permeability and peripheral vasodilation. To compensate, there will be an increase in HR and BP, and a decrease in QT.[21–24] This was also found in our study before gastrointestinal and pulmonal symptoms occurred, but the DBP decreased before skin/mucous membrane symptoms. This might be due to stress or an acute rise in blood pressure, which causes stiffening of the large arteries increasing SBP further and decreasing DBP.[25–28] Ruiz-Garcia et al. showed comparable results.[5] In our study, before cardiovascular symptoms occurred, HR, DBP, and SBP decreased significantly. This might be explained due to a failed compensation for fluid redistribution. During a severe allergic reaction, due to the high vascular permeability and high loss of fluid, the BP will decrease while the HR will increase to compensate.[21–23,29,30] This is also observed in **Figure 1**: around 15 minutes before cardiovascular symptoms occurred, both the SBP and DBP decreased and HR increased, but only a few minutes before the cardiovascular symptoms, the HR stopped increasing and abruptly decreased. However, it is important to note that only two patients experienced a cardiovascular event. More patients are needed to draw firm conclusions.

The release of histamine and pro-inflammatory cytokines can also lead to bronchoconstriction and laryngeal edema which causes a decrease in SpO2. Normally, a decrease in SpO2 below 95% will lead to an increase in RR.[31] We showed that before pulmonary symptoms occurred, indeed the SpO2 decreased, but we noticed a decrease of the RR which we cannot explain. However, after the pulmonary symptoms, the RR compensated and increased. Before cardiovascular symptoms occurred, the respiratory parameters changed as expected (decrease in SpO2; increase in RR) which is also observed in studies about anaphylaxis.[29,30,32] The significant increase in RR, and therefore SpO2, before gastrointestinal symptoms may be due to pain, stress or hyperventilation.[33,34]

It is important to note that multiple reactions often occur rapidly one after the other. We tried to incorporate this by investigating the differences in parameters before the last dose leading to the discontinuation of the OFC.

Differences in physiological parameters around doses that increased before an objective allergic reaction

Visual exploration of parameters over time showed that the sum of the rolling standard deviation seems to fluctuate around each dose for both patients with and without an objective allergic reaction. This might be due to anxiety. Asking patients to ingest a food that has previously been avoided and considered dangerous by a patient may cause stress and anxiety about the possibility of an allergic reaction occurring during the OFC.[35,36] During an acute stressful situation, an increase is seen in the RR and HR[25–27], which are also the parameters that showed the highest variations during the given doses in our present study. Healthcare professionals need to be aware of this.[35]

Although both patients with and without objective symptoms showed fluctuations over time, differences were seen between the two groups: the fluctuations over time up to the last ingested dose showed an increased pattern for patients with an objective allergic reaction while this increase was not that obvious for patients without an objective allergic reaction. These differences makes it possible to differentiate an objective allergic reaction from non-objective allergic reactions.

Strengths and limitations

The main limitation of this pilot study was the small sample size. However, continuous measurement of seven parameters yielded a detailed dataset, providing the opportunity to explore early physiological changes, develop predictive models for OFCs, and offer valuable insights for future research.

CONCLUSION

We have shown that different combinations of multiple physiological parameters over time measured by continuous monitoring are related to different objective allergic symptoms. It might be possible to predict an objective allergic reaction that would lead to discontinuation of an OFC during a period before the dose that leads to discontinuation.

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8

Allergenicity of eight soy products: allergy to soy dairy is most frequent and most severe

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Under review

Background

Soy is an attractive protein source that is commonly used in plant-based foods. There are several soy products on the market, all processed in a different way. The objective of this study was to investigate differences in frequency and severity of soy allergy in response to eight different soy products.

Methods

Adult patients with a soy allergy, i.e. with a suggestive history and sensitization to soy or a positive food challenge, were included. Eight soy products were investigated: soy dairy, tofu, tempeh, soybean, meat substitutes based on soy, soy sauce, bread containing soy flour and soy containing biscuit "Biscoff".

Results

Sixty patients were included. Soy dairy most commonly caused symptoms (94.4% of patients), followed by tofu (45.8%), soybean and tempeh (both 41.2%), meat substitute (37.5%), soy sauce (28.3%), bread (16.7%), and Biscoff (16.6%). There was a significant difference in the proportion of patients reporting symptoms ($p<0.001$) to the eight soy products. Of the patients who reported symptoms to soy dairy, 52.9% did not report symptoms to other soy products. Regarding severity, Biscoff least often caused severe symptoms. Compared to Biscoff, patients who consumed soy dairy had the highest chance to report severe symptoms (OR=31.3), followed by tofu (OR=4.0), meat substitute (OR=3.6), soybean (OR=3.3), tempeh (OR=3.3), soy sauce (OR=2.9), and bread (OR=1.6).

Conclusion

There is a significant difference in frequency and severity of food allergy between the eight different soy products. Soy dairy is responsible for most reactions as well as most severe reactions. When symptoms to soy dairy are reported, this does not imply a reaction to other soy products. Therefore, if there is a clinical response to soy dairy, it is probably not necessary to avoid all soy products.

INTRODUCTION

Both protein consumption and the world's population are increasing which leads to an urgent demand for sustainable protein sources.[1,2] Soy is an attractive protein source and is commonly used in plant based foods. In the last decade, consumption of soy foods in Western countries has grown with the increase in veg(etari)an lifestyle.[3,4] It is expected that the consumption of soy products will increase even more in the future, which may cause a rise in soy allergy.

Many different products are made of soy, all with their own type of processing.[5] The allergenicity of soybean and various products thereof is poorly characterized[5] and therefore it is unknown whether there is a difference in frequency and severity of food allergy to different soy products.

The severity of a soy allergy can vary from mild to anaphylactic reactions. While pollen food cross-reactivity commonly presents with mild symptoms, severe reactions to soy dairy have been reported repeatedly in patients with birch pollen-related soy allergy (BPSA).[6–9] In these patients, an allergy to soy is caused by the cross-reactive soy allergen Gly m 4, the soy homologue of the major birch pollen allergen Bet v 1.[9]

Due to the increasing consumption of soy as a dietary protein source, there is a need to further investigate the allergenic properties of different processed soy products, both in terms of frequency and severity of reported symptoms. The primary aim of this study was to investigate this for eight different commonly consumed soy products with varying processing characteristics. The secondary aims was to identify predictors for severe soy allergy.

METHODS

Patient selection

Adult patients who visited the outpatient clinic of the Dermatology/Allergology Department of the University Medical Center Utrecht (UMCU), the Netherlands, with reported symptoms to soy within 2 hours and/or positive food challenge to soy and sensitization to at least one of the sensitization tests to soy (henceforward patients with a soy allergy), between October 2021 and September 2023 were included.

This study was approved by the Medical Research Ethics Committee of the UMCU (protocol number 22-983).

Data collection

Clinical questionnaire

Physicians routinely collected clinical information during an outpatient clinic visit, using a standardized questionnaire. Information was collected on patient demographics (age, sex), allergic comorbidities (allergic rhinoconjunctivitis [AR], allergic asthma [AA], atopic dermatitis [AD]), reactions to one or more different soy products and their severity. The following relatively commonly ingested soy products with different processing methods were evaluated: soy dairy, tofu, tempeh, soybean, meat substitutes based on soy protein (henceforth meat substitutes), soy sauce, bread containing soy flour (henceforth bread) and the soy containing biscuit "Biscoff" (henceforth Biscoff).

Sensitization tests

Sensitization was measured by skin prick tests (SPT), prick-to-prick (PTP) and specific IgE (sIgE) tests. SPT (ALK, Madrid, Spain) were performed to birch pollen and soy extract and PTP to soymilk, tofu and/or tempeh. sIgE (ImmunoCAP, ThermoFisher Scientific, Uppsala, Sweden) was measured to birch pollen, Bet v 1, soy extract, Gly m 4, Gly m 5, and Gly m 6. A mean wheal diameter ≥ 3 mm and IgE levels ≥ 0.10 kU_A/L were considered positive, respectively.

Severity of symptoms

Severity of symptoms was classified using the oFASS-3 score, which is divided into three categories: oFASS-3 score 1 consists of symptoms of the oral cavity (i.e. mild symptoms), score 2 consists of symptoms of the skin, nose, eyes, the digestive system and/or uterus with or without score 1 symptoms (i.e. moderate symptoms) and score 3 consists of symptoms of the respiratory and/or cardiovascular system with or without score 1 and/or 2 symptoms (i.e. severe symptoms).[10]

Statistical analysis

Continuous variables were described as mean with standard deviation (SD) when normally distributed or as median with interquartile range (Q1, Q3) when not normally distributed. Categorical variables were described with frequency and percentage.

The frequency of reported symptoms (percentage of patients) of the eight soy products were descriptively compared. To account for the fact that we assessed symptoms to eight different food types for each patient (i.e. multiple outcomes within patients), the frequency of symptoms was statistically compared between soy products using a random effect logistic regression with complaints yes/no as outcome, soy product type as independent variable and a random intercept on patient level. To compare the frequency of severe food allergy, the same approach was used, with moderate to severe symptoms yes/no as outcome.

To investigate factors that are associated with a severe reaction to the different soy products, univariate and multivariate logistic regression analysis (including variables statistically significant in the univariate analysis or deemed clinically relevant (ImmunoCAP Gly m 4) was performed per soy product. A backward selection process was used removing variables one-by-one based on statistical significance, until all variables in the model had a p-value < 0.2 to arrive at the final model.

Analyses were performed using IBM SPSS Statistics version 27 (2020, IBM, Armonk, NY, United States of America). P values ≤0.05 were considered statistically significant unless stated otherwise.

RESULTS

Patient characteristics

A total of 60 patients were included of which 37 (61.7%) were female and 51 (85.0%) had allergic rhinitis to birch pollen. The patient characteristics are shown in **Table 1**. Most patients reported symptoms to soy dairy and the median severity of all soy product together on a scale from 1-3 was 2.1.

Most patients were sensitized to ImmunoCAP Gly m 4 (90.7%) and PTP soy dairy (80.6%). Notably, ImmunoCAP to soy extract was only positive in 59.6% of patients.

Soy dairy is the most common and most severe soy allergy

Symptoms were most frequently reported to soy dairy (94.4%), followed by symptoms to tofu (45.8%), soybean and tempeh (both 41.2%), meat substitute (37.5%), soy sauce (28.3%), bread (16.7%), and Biscoff (16.6%) **(Table 1 and Figure 1a**). Reported symptoms between the various soy products differed significantly (p < 0.001 for variable soy product in analysis). Compared to Biscoff, patients who consumed soy dairy had the highest chance (OR=43.9 [95% CI 16.5 – 116.9]) to react and soy sauce and bread the lowest chance (OR=1.8 [95% CI 0.7 – 4.7) and OR=1.0 [95% CI 0.4-2.7], respectively) (**Table 2**).

Of the patients who reported symptoms to soy dairy, 52.9% did not report symptoms to other soy products (**Figure 2 and Table S.1)**. When they did report symptoms to other soy products, this was most often to tofu (45.0%) and least often to Biscoff (17.9%). On the contrary, almost all patients who reported symptoms to a soy product other than soy dairy, reported symptoms to multiple soy products, of which with soy dairy was most common (81.8% - 100%) and bread and Biscoff least common (17.9% - 60.0%).

Table 1. Patient characteristics of soy allergic patients

	Population N=60	
Age in years; mean (±SD)	34.4	(14.1)
Female	37	(61.7)
Anamnestic atopic comorbidities		
Allergic rhinitis - birch pollen	51	(85.0)
Asthma	25	(41.7)
Atopic dermatitis	32	(53.3)
Reported symptoms to*		
Soy dairy	51/54	(94.4)
Tofu	11/24	(45.8)
Soybean	14/34	(41.2)
Tempeh	7/17	(41.2)
Meat substitute	12/32	(37.5)
Soy sauce	15/53	(28.3)
Bread	8/48	(16.7)
Biscoff	6/36	(16.6)
Median severity (±Q2-Q3)**	2.1	(2.0-3.0)
Positive SPT/PTP*		
SPT birch pollen	52	(91.2)
SPT soy extract	24	(49.0)
PTP soy milk	29	(80.6)
PTP tofu	21	(56.8)
PTP tempeh	12	(33.3)
Positive ImmunoCAP***		
Birch pollen	15	(88.2)
Bet v 1	9	(90.0)
Soy	31	(59.6)
Gly m 4	49	(90.7)
Gly m 5	3	(12.5)
Gly m 6	5	(20.8)

*All measurements are in N (%) unless otherwise specified. * The percentage given in brackets is the percentage of the total number of patients that reported symptoms/number of patients that ingested the food. ** Scale 1 (mild symptoms) to 3 (severe symptoms) *** The percentage given in brackets is the percentage of the total number of patients that reported symptoms/number of patients that were tested.*

Soy dairy caused by far the most severe symptoms, with more than half of the patients reporting severe symptoms to soy dairy and almost a third reporting moderate symptoms **(Figure 1b**). The other soy products caused severe (2.1% - 12.5%) and moderate (8.3% - 28.1%) symptoms less commonly. The frequency of moderate and severe symptoms between the soy products differed significantly ($p < 0.001$). Compared to Biscoff, patients who consumed soy dairy had the highest chance to report moderate or severe symptoms

(OR=31.3 [95% CI 9.1 – 107.8]) and soy sauce and bread had the lowest chance (OR=2.9 [95% CI 0.9 – 9.6] and OR=1.6 [95% CI 0.4-5.8], respectively) (**Table 2**).

Of the patients who reported severe symptoms to soy dairy, 28.6% also reported severe symptoms to other soy products. This was mostly to tempeh (33.3%). Of the patients who reported moderate symptoms to soy dairy, 53.3% also reported moderate symptoms to other products. This was mostly to meat substitute (44.4%) (**Table 3**).

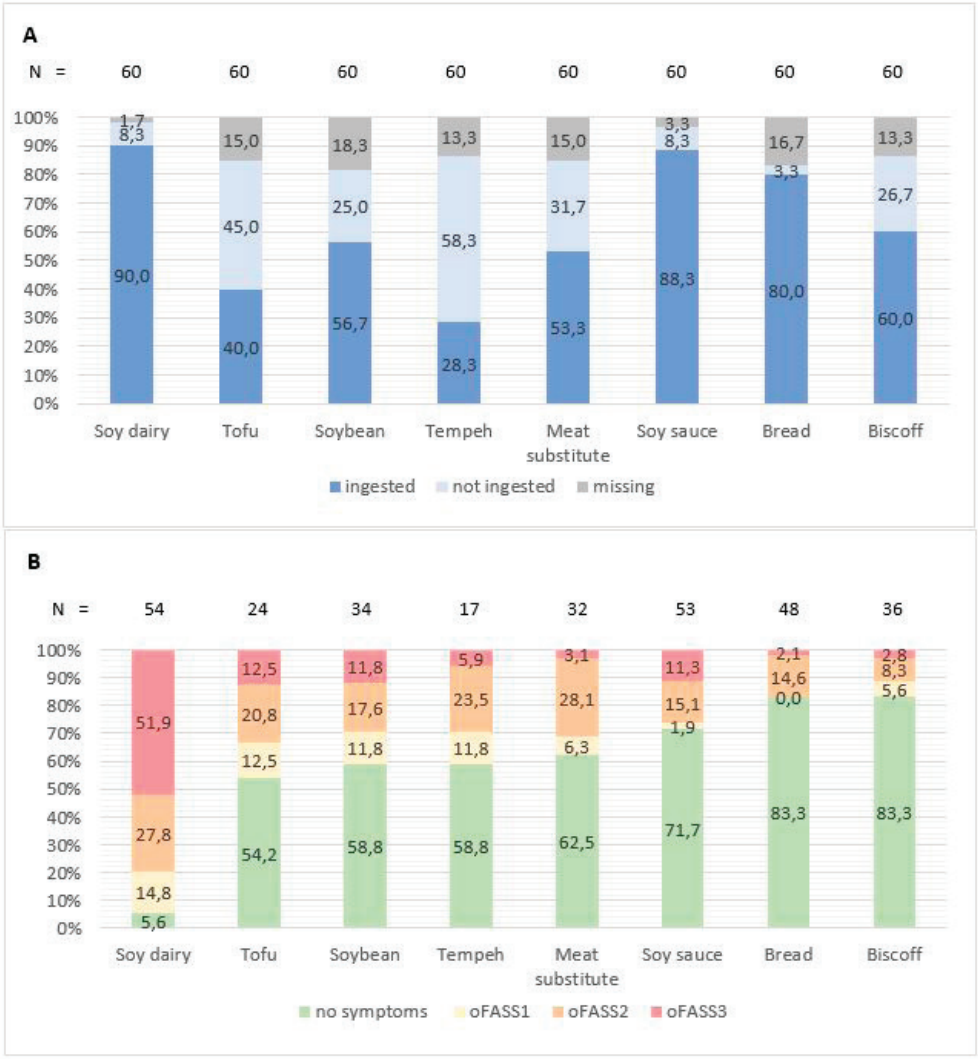


Figure 1. Overall clinical response to all the different soy products for (A) all patients and (B) patients that ingested the soy products

Table 2. Odds ratios for frequency of allergy diagnosis and frequency of moderate/severe allergy

	Frequency of allergy diagnosis*		Frequency of moderate/severe allergy**	
	Odds ratio	(95% CI)	Odds ratio	(95% CI)
Soy dairy	43.9	(16.5 – 116.9)	31.3	(9.1 – 107.8)
Tofu	4.1	(1.2 – 13.7)	4.0	(1.0 – 15.4)
Soy bean	3.3	(1.1 – 9.8)	3.3	(0.9 – 12.0)
Tempeh	3.3	(0.9 –12.6)	3.3	(0.8 –14.6)
Meat substitute	2.8	(0.9 – 8.3)	3.6	(1.0 – 13.2)
Soy sauce	1.8	(0.7 – 4.7)	2.9	(0.9 – 9.6)
Bread	1.0	(0.4 – 2.7)	1.6	(0.4 – 5.8)
Biscoff	Reference	NA	Reference	NA

** Patients with symptoms versus no symptoms to soy products; ** Patients with moderate/severe symptoms versus patients with no/mild symptoms*

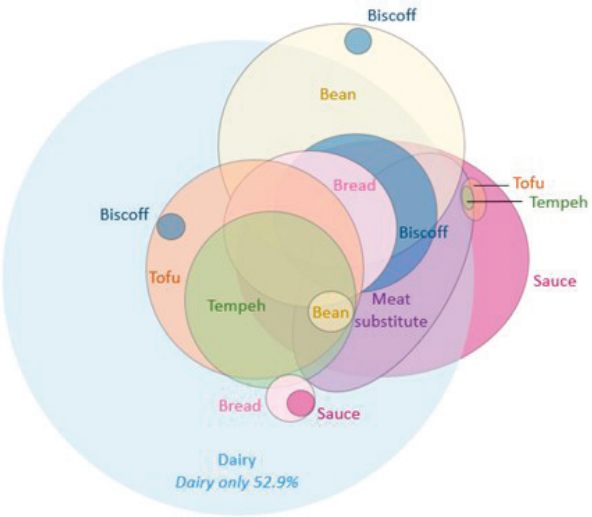


Figure 2. Overview of co-allergies of the different soy products

Table 3. Reported symptoms per soy product per patient sorted from most to least severe

Soy Dairy	Soy sauce	Soy bean	Tofu	Meat substitute	Bread	Tempeh	Biscoff
3	3	3	NI	0	0	NI	1
3	3	1	NI	2	0	NI	NI
3	3	NI	NI	2	2	NI	3
3	3	NI	NI	NI	3	NI	0
3	3	NI	NI	NI	0	NI	NI
3	0	3	0	NI	0	NI	0
3	0	2	2	NI	2	NI	0
3	0	0	3	3	0	3	NI

Table 3. Reported symptoms per soy product per patient sorted from most to least severe (*continued*)

Soy Dairy	Soy sauce	Soy bean	Tofu	Meat substitute	Bread	Tempeh	Biscoff
3	0	0	3	0	0	NI	0
3	0	0	0	2	0	NI	0
3	0	0	0	0	0	NI	0
3	0	0	0	0	-	NI	-
3	0	0	0	NI	0	0	0
3	0	0	NI	0	0	NI	0
3	0	0	NI	0	0	NI	0
3	0	0	NI	NI	0	NI	NI
3	0	0	NI	NI	NI	NI	NI
3	0	NI	0	0	0	0	0
3	0	NI	NI	NI	0	NI	0
3	0	NI	NI	NI	0	NI	NI
3	0	NI	NI	NI	0	NI	NI
3	0	-	0	0	-	NI	NI
3	0	-	NI	0	0	NI	NI
3	0	-	-	-	0	-	0
3	0	-	-	-	-	-	-
3	NI	0	NI	NI	0	NI	0
3	NI	NI	NI	NI	0	NI	0
3	NI	NI	NI	NI	0	NI	0
2	2	2	2	2	2	2	2
2	2	2	NI	2	2	NI	2
2	2	NI	2	NI	2	2	0
2	2	-	-	-	-	-	-
2	1	3	1	2	0	1	NI
2	0	1	NI	NI	0	NI	0
2	0	0	3	0	0	0	2
2	0	0	0	2	0	0	NI
2	0	0	0	0	2	0	0
2	0	0	0	0	0	0	-
2	0	0	NI	0	0	NI	0
2	0	NI	NI	0	0	NI	0
2	NI	-	NI	-	-	NI	NI
2	NI	-	-	-	NI	-	NI
2	-	-	-	-	-	-	-
1	0	1	0	0	0	0	0
1	0	0	1	NI	0	0	NI
1	0	0	-	-	0	-	-

Table 3. Reported symptoms per soy product per patient sorted from most to least severe (*continued*)

Soy Dairy	Soy sauce	Soy bean	Tofu	Meat substitute	Bread	Tempeh	Biscoff
1	0	NI	NI	1	0	NI	0
1	0	NI	NI	NI	2	2	NI
1	0	-	1	1	-	1	0
1	0	-	-	-	0	-	-
1	-	-	-	-	-	0	-
0	3	0	NI	NI	0	NI	NI
0	2	2	NI	2	0	NI	0
0	0	2	NI	0	0	NI	0
NI	2	0	0	0	-	0	0
NI	2	NI	2	2	0	2	0
NI	2	NI	2	0	0	NI	0
NI	0	2	NI	0	0	NI	0
NI	0	1	0	0	0	NI	0
-	0	3	-	NI	-	-	1

Legend: 0 = no symptoms; 1 = mild symptoms, 2 = moderate symptoms, 3 = severe symptoms, NI = not ingested, - = missing

Sensitization to soy extract was inversely associated with severe soy dairy allergy

Due to the small number of patients who reported symptoms to the different soy products, only the soy dairy group was large enough and used to predict severity. In total, 23 patients had mild/moderate symptoms to soy dairy and 28 had severe symptoms. **Table 4** shows the results of the univariable analysis on severe soy dairy symptoms. 'Atopic dermatitis' and 'sensitization to soy extract' were negatively associated with severe soy dairy allergy.

The binary variables 'atopic dermatitis', 'sensitization to CAP soy extract' and the continuous variable 'CAP Gly m 4' were used in a multivariable logistic regression analysis. In this model, sensitization to CAP soy extract was the only variable significantly inversely associated with severity with an OR of 0.214 (95% CI 0.06-0.8).

DISCUSSION

To our knowledge, this is the first study that investigated frequency and severity of soy allergy to as many as eight different soy products. We showed that there are significant differences between frequency and severity of allergy to the different soy products, with

soy dairy causing the most frequent and severe symptoms and bread and Biscoff causing the least frequent and least severe symptoms.

Frequency of allergy to different soy products differs

This study showed that of the eight different soy products to which a patient might be allergic, soy dairy most commonly caused symptoms, followed by symptoms to tofu, soybean, tempeh, meat substitutes, soy sauce, bread, and "Biscoff". It is notable that almost all patients reported symptoms to soy dairy. A possible explanation for the difference in the frequency of allergy to the different soy products could be differences in consumption. For all the soy products in this study except for soy sauce, the EPIC study[11] recorded consumption data in the Netherlands. The soy

Table 4. *Univariate analysis of different variables to predict severe soy dairy allergy*

	Mild/moderate (N=23)		Severe (N=28)		p-value	OR (95% CI)	
Age in years; mean (±SD)	31.7	(11.7)	39.8	(15.4)	0.052	1.0	(1.0 - 1.1)
Female	14	(60.9)	18	(64.3)	0.802	1.2	(0.4-3.6)
Anamnestic atopic comorbidities							
Allergic rhinitis - birch pollen	19	(82.6)	24	(85.7)	0.762	1.3	(0.3-5.7)
Asthma	10	(43.5)	12	(42.9)	0.964	1.0	(0.3-3.0)
Atopic dermatitis	16	(69.6)	11	(39.3)	**0.034**	**0.3**	**(0.09-0.9)**
Reported symptoms to*							
Tofu	6	(60.0)	3	(30.0)	0.185	0.3	(0.0-1.8)
Soybean	5	(41.7)	4	(26.7)	0.414	0.5	(0.1-2.6)
Tempeh	5	(41.7)	1	(33.3)	0.793	0.7	(0.0-10.0)
Meat substitute	6	(50.0)	4	(30.8)	0.330	0.4	(0.1-2.3)
Soy sauce	5	(23.6)	5	(20.0)	0.621	0.7	(0.2-2.8)
Bread	5	(29.4)	3	(12.5)	0.189	0.3	(0.1-1.7)
Biscoff	3	(17.6)	2	(7.7)	0.332	0.4	(0.1-2.6)
Severe symptoms to other soy product	2	(10.0)	8	(28.6)	0.134	3.6	(0.7-19.2)
Positive SPT/PTP*							
SPT birch pollen	18	(85.7)	26	(93.6)	0.220	4.3	(0.4-45.1)
SPT soy extract	12	(66.7)	8	(36.4)	0.061	0.3	(0.1-1.1)
PTP soy dairy	15	(78.9)	9	(81.8)	0.850	1.2	(0.2-7.9)
Positive ImmunoCAP*							
Soy extract	15	(75.0)	10	(40.0)	**0.022**	**0.2**	**(0.1-0.8)**
Gly m 4	17	(85.0)	26	(96.3)	0.203	4.6	(0.4-47.8)
Gly m 5	2	(22.2)	0	(0)	0.999	0.0	NA
Gly m 6	2	(22.2)	2	(16.7)	0.749	0.7	(0.1-6.2)

All measurements are in N (%) unless otherwise specified. * The percentage given in brackets is the percentage of the total number of patients that reported symptoms/were tested.

product that most commonly caused symptoms in our study (soy dairy) was also the most frequently consumed soy product. Soy flour was the least consumed food product, and this corresponds with our finding that this was one of the least common soy products causing an allergy. So, the frequency of allergy to certain soy products could be associated with the frequency of consumption. However, two studies showed that there was no correlation between soy consumption and soy sensitization.[12,13] Further studies must elucidate the association between consumption and allergy to different soy products. Knowledge on this topic is important because in the future, as the world's population grows, alternative protein sources such as soy will be important and an increase in soy consumption is to be expected.

Frequency of severity of allergy to different soy products differs

There was a significant difference in severity of symptoms to the various soy products: soy dairy clearly caused the most severe symptoms, followed by tofu, meat substitutes, soybeans, tempeh, soy sauce, bread and Biscoff, all to a much lesser extent.

A potential explanation for the observed difference in severity could be attributed to differences in processing. The types of processing that have been implicated in influencing allergenic properties are: heating, fermentation (including endogenous enzymatic hydrolysis, enzymatic and acid hydrolysis), physical treatments (such as high pressure processing or extrusion), the use of preservatives, changes in pH, or combinations of these.[14,15] Allergenicity could be reduced by food processing and the combination of multiple processing types is more effective than a single method to reduce soybean allergenicity, especially fermentation combined with other processing types.[5,16] However, the evidence is limited because there are no high-quality clinical relevant studies performed in soy allergic patients.[5] Although the processing types for soy products are difficult to compare, based on Verhoeckx et al. the degree of food processing from least to most processed would be estimated as follows: 1) soybean, 2) soy dairy, 3) tofu, 4) soy sauce and tempeh, 5) soy flour and meat substitutes.

In addition to processing, the amount of Gly m 4 protein in soy products could also play a role in the severity. Gly m 4, the soy pathogenesis-related protein, has been associated with severe food adverse reactions.[9,17,18] Yoshimitsu et al. showed a large difference in Gly m 4 levels with 309-421 µg/g in soybean, 45-67 µg/g in soymilk, and 2.7 - 45 µg/g in roasted soy flour.[19]

Comparing the degree of processing and the level of Gly m 4 with our established severity ranking, it appears that this is not exactly the same. Comparing the processing steps is therefore important. Unfortunately, the exact processing steps are not always known and differ between food producers. This makes it difficult to draw conclusions. However, soy dairy was found in our study to most commonly cause severe symptoms and this was

also one of the least processed (soaking in water and heating) products with the second highest amount of Gly m 4. Soybeans, which contained the highest Gly m 4 levels[19], did not cause the most severe symptoms in our study. This might be explained by differences in food processing techniques, which was not taken into account in our study. The advice is not to eat raw soybeans because of the presence of the toxic substance lecithin which can cause intestinal complaints.[20] For that reason, soybeans are generally eaten cooked. The amount of Gly m 4 in soybeans can be drastically reduced by heating, which was shown by Mittag et al.[21] This might explain that allergic reactions to soybean are not often severe. However, as the reduction in allergenicity varies depending on the type of allergen and only a few studies have investigated the effect of different processing methods, more research is needed.

Soy dairy most frequently severe when sensitized to Gly m 4

In our study, 90.7% of the patients recognized Gly m 4. The location of our center in a birch endemic area explains this high sensitization rate. Among patients who recognized Gly m 4, 60.5% reported severe symptoms to soy dairy **(Table S2**). The association between birch pollen-related soy allergy (BPSA) and severe symptoms, especially to soy dairy, is frequently described in the literature.[6–9] Although birch pollen-related food allergy is most commonly mild, BPSA is an exception as it frequently causes severe reactions. Possible explanations for why soy dairy more commonly causes severe symptoms than other birch pollen-related foods are the possibly more stable character of Gly m 4, a higher ingested amount, and a high-protein matrix that protects against degradation and favors a severe response.[9,22–24]

The association between primary soy allergy (Gly m 5 and Gly m 6) and having severe symptoms is mentioned in literature as well.[23,25,26] As in our study only 3 and 5 patients were sensitized to Gly m 5 and Gly m 6, respectively, no conclusion can be drawn about possible differences between patients who are sensitized to Gly m 5/6 and those who are sensitized to Gly m 4 regarding symptom severity of the eight soy products.

With a soy dairy allergy, it may be possible to eat other soy products

Our study showed that a reaction to soy dairy does not imply a reaction to other soy products: more than half of the patients who reported symptoms to soy dairy did not report symptoms to other soy products. Therefore, when patients are diagnosed with a soy dairy allergy, they should not be advised to avoid all soy products. Biscoff, bread and soy sauce are least likely to cause an allergy, and if they do cause a reaction, it is likely to be the least severe. We would therefore advise reintroducing these products carefully into the diet first.

CAP soy extract may be associated with less severe soy dairy allergy

The only factor that was inversely associated with a severe soy dairy allergy was CAP soy extract. Berneder et al. state that CAP soy extract is highly specific for Gly m 5 and 6, whereas Gly m 4 is not.[9] Because in our study CAP soy extract was inversely related to severity, this would mean that patients sensitized to Gly m 5 and 6 would have less severe soy dairy allergy and patients sensitized to Gly m 4 more severe symptoms. However, we only showed a trend in the univariable analysis that more patients with mild symptoms were sensitized to Gly m 5 and 6. This was probably due to the small numbers of patients in whom sIgE to Gly m 5 and 6 was measured. It would be interesting to investigate this in more patients where sensitization was measured.

Gly m 4 level was not a predictor of severe soy dairy allergy in our model. Although no association was found between Gly m 4 levels and severity of the reaction, we found that 60.5% of the patients with sensitization to Gly m 4 had severe soy dairy symptoms compared to 25.0% of the patients who were not sensitized to Gly m 4. Berneder et al. also showed that sensitization to Gly m 4 tends to induce moderate to rather severe symptoms, with no association between the severity of soy allergic symptoms and IgE levels to Gly m 4.[9]

Sensitization to soy extract alone is not sufficient to diagnose soy allergy

This study showed that sensitization tests for soy extract alone are not sufficient to diagnose soy allergy. Both SPT to soy extract and ImmunoCAP to soy extract showed low sensitization rates (positive in 49.0% *versus* 59.6%). The low sensitization rates to soy extract could be explained by the underrepresentation of Gly m 4 protein in the soy extract compound.[27] ImmunoCAP Gly m 4 was the best performing test (positive in 90.7%), followed by PTP to soy milk (positive in 80.6%). It is recommended to perform ImmunoCAP to soy extract and Gly m 4, and PTP with soy milk in patients with a suspected soy allergy.

Limitations and strengths

The gold standard for diagnosing soy allergic patients is an oral food challenge. In this study, diagnosis was mostly based on history and sensitization to soy, and a food challenge was only performed when the diagnosis was uncertain or when specific food products needed to be evaluated. Although an OFC is the gold standard, in clinical practice food allergy diagnosis is often based on suggestive history and corresponding sensitization. Another limitation is the high number of patients that had not ingested one or more of the eight soy products. However, due to the prospective design and the standardized questionnaire we got as much data as possible from our patients.

Strengths of this study are the large number and variety of soy products that were compared and the relatively large study population.

CONCLUSION

There is a difference in frequency of symptoms and frequency of moderate to severe symptoms of food allergy between different soy products. Soy dairy most commonly caused reactions and most often caused severe reactions. When symptoms to soy dairy are reported, it is probably not necessary to avoid all soy products.

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9

Birch pollen-related food allergy: occasionally severe, yet home-based oral food challenges are safe

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Background

Birch pollen-related food allergy (BPFA) is the most common food allergy in Northern and Central Europe, but studies in highly-characterized patients are limited. This study describes detailed symptoms and reaction severity during oral food challenges (OFC) and home-based oral food challenges (HOFC) in BPFA.

Methods

Included patients were part of the Itulazax Food Allergy/Allergic Rhinitis (Itula-FAR) study. Adults with birch pollen allergy, sensitized to PR-10 components of the culprit food(s) but not to non-PR10 components, and a positive OFC or HOFC were included. Doctor's diagnosed severity was assessed using the oFASS-5 severity score and patient-perceived severity by numeric rating scale (NRS). Health-related quality of life (HRQL) was evaluated with the Food Allergy Quality of Life Questionnaire-Adult Form (FAQLQ-AF).

Results

Thirty-eight patients were included, with a mean FAQLQ-AF total score of 3.3, indicating moderate HRQL impairment. Patients reported most frequently symptoms to fruit (94.7%), followed by nuts (76.3%), vegetables (52.6%), and legumes (36.8%). Ninety-three challenges were positive, and in 84.2%, patients also had other than oropharyngeal symptoms. NRS scores across organ systems ranged from 3.4 to 4.4, indicating moderate impairment. Based on oFASS, reactions were mild in 43.0%, moderate in 44.1%, and severe in 12.9%. However, severe reactions classified by oFASS did not reach the most extreme end of the severity spectrum as no epinephrine was used during OFC/HOFC. Among hazelnut challenges, raw hazelnut provoked most commonly severe reactions (16.0%), followed by Nutella (8.0%) and roasted hazelnut (4.0%). Overall, 45% of challenges were performed at home. Of these, 8.9% were classified as severe by oFASS, but were perceived as mild by patients (mean NRS: 3.2).

Conclusions

While BPFA is generally mild, over 80% of patients experience symptoms beyond the oropharynx, and 12.9% of reactions are classified as severe. HOFC appears safe in this patient group, suggesting its potential to reduce hospital resource utilization.

INTRODUCTION

Birch pollen allergy is one of the most common pollen allergies in Northern- and Central Europe. Up to 70-80% of affected patients also experience allergic symptoms to certain fruits, nuts and vegetables.[1,2] These symptoms are due to IgE cross-reactivity between the major birch pollen allergen, Bet v 1, and it's homologues in food[3] and is known as 'birch pollen-related food allergy' (BPFA).[4–7]

Patients with BPFA typically report mild oropharyngeal symptoms. However, more severe allergic reactions such as respiratory or cardiovascular symptoms can occur.[4,5,8–10] Studies investigating symptom severity in BPFA using oral food challenges (OFCs), the diagnostic gold standard, are scarce. Furthermore, comprehensive immunological characterization, particularly of those sensitized exclusively to PR-10 allergens and not to non-PR-10 allergens, is often lacking. Consequently, existing data may be biased, since many studies do not clearly distinguish BPFA from primary food allergy. Greater diagnostic precision, including molecular sensitization profiling, is essential for accurate interpretations and management.

Patient-reported outcome measurements (PROM) are valuable for assessing symptoms and quality of life. They can complement traditional clinician measures like severity scoring during OFCs and potentially improve patient engagement by capturing real-world concerns and supporting shared decision-making.[11] However, PROM data in BPFA are limited. Some studies have used the visual analogue scale (VAS) to assess the subjective experience of symptoms, but results ranging widely, and BPFA confirmation is often unclear.[12–15]

OFCs help to confirm food allergy, determine thresholds, and identify symptoms elicited.[16] With increasing healthcare pressure, home-based oral food challenges (HOFC) may offer a safe, practical alternative for selected patients with suspected food allergy, especially for those with BPFA since majority will have mild reactions. HOFCs could reduce the burden on clinical resources while empowering patients to better understand their own symptom patterns. However, as far as known, no studies have evaluated HOFCs.

This study aims to characterize patients with BPFA by examining detailed symptoms and severity during OFCs and HOFCs, as well as patients' perceptions of these symptoms.

METHODS

Subject selection

Part of the patients were enrolled in the Itulazax Food Allergy/Allergic Rhinitis (ItulaFAR) outpatient clinic study. Patients aged 18-65 years who visited the Department of Dermatology and Allergology at the University Medical Center Utrecht (UMCU), the Netherlands, were recruited between September 2022-December 2024. Eligible patients had a diagnosis of birch pollen-induced allergic rhinitis (AR), were willing to initiate birch pollen sublingual immunotherapy (SLIT), and had concomitant BPFA.

BPFA was defined as follows:

1. Presence of symptoms with sensitization to birch pollen extract and/or Bet v 1 in at least one test (skin prick test [SPT][ALK, Madrid, Spain] or specific IgE [sIgE]), and;
2. Positive challenge for at least one of the following foods: almond, apple, carrot, cherry, hazelnut, kiwi, peach, plum, walnut, or soymilk, with sensitization to that food's extract (via SPT, prick to prick test [PTP], or ImmunoCAP [ThermoFisher Scientific, Uppsala, Sweden]). When commercially available, patients also had to be sensitized to the associated PR-10 component (e.g., Cor a 1 for hazelnut), without sensitization to non-PR-10 components (e.g., Cor a 9), and;
3. Confirmed sensitization to customized immunoblots (EUROLINE, EUROIMMUN, Lübeck, Germany) for PR-10 mix (Bet v 1, Que a 1, Mal d 1, Jug r 5, Ara h 8), and absence of sensitization to lipid transfer protein (LTP) mix (Pru p 3, Tri a 14, Pha v 3, Lac s 1, Mal d 3, Jug r 3, Art v 3, Cor a 8, Ara h 9), nut seed storage protein (SSP) mix (Ana o 3, Jug r 1, Ber e 1) and peanut SSP mix (Ara h 1, 2, 3, 6, 7).

The study was approved by the local ethics committee (protocol number 22-819), and all patients provided written informed consent.

Data collection

Clinical and demographic data

Data on patient demographics (age, sex), allergic comorbidities (allergic rhinitis, asthma , atopic dermatitis), clinical history, frequency and severity of symptoms to BPFA-related foods, FA-QoL, sensitization, OFCs, and HIs were collected.

Questionnaires

The Food Allergy Quality of Life Questionnaire – Adult Form (FAQLQ-AF) was administered two weeks after the challenge to evaluate impact on HRQL.[17] The FAQLQ-AF encompasses 29 questions across four domains, 1) allergen avoidance and dietary restrictions,

2) emotional impact, 3) risk of accidental exposure, and 4) food allergy related health. Each question scored between 1 (not troubled) and 7 (extremely troubled). [17]

Sensitization tests

For all patients, total IgE was measured, as well as sIgE to birch pollen, Bet v 1, almond, apple, Mal d 1, apricot, carrot, celery, Api g 1, cherry, hazelnut, Cor a 1, Cor a 9, Cor a 14, kiwi, Act d 8, peach, Pru p 1, Pru p 3, peanut, Ara h 2, Ara h 6, Ara h 8, pecan, soy, Gly m 4, Gly m 5, Gly m 6, and walnut (ImmunoCAP and/or immunoblot). SPT and/or PTP were performed for birch pollen, almond, apple, bell pepper, carrot, celery, cherry, hazelnut, kiwi, walnut, peanut, peach, pecan, soy, and tomato. For soy-related symptoms, PTP was also performed with soymilk, tofu, and tempeh. Positive results were defined as sIgE levels ≥ 0.35 kU_A/L, or a mean wheal diameter ≥ 3 mm, respectively.

Furthermore, to accurately screen and select eligible patients, at baseline, sIgE to ImmunoCAP Cor a 9, Cor a 14, Pru p 3, Ara h 2, Ara h 6, Gly m 5, and Gly m 6 and immunoblots with PR-10-, LTP-, nut SSP-, and peanut SSP- mixes were measured for all patients. Immunoblot analyses followed manufacturer instructions, using EUROBlotOne system and EUROLineScan software. Results ≥ EAST class 1 were considered positive.

Oral food challenge and home introduction

OFCs and HOFCs (henceforward referred to as 'challenges' when mentioned together) were performed following the local protocol, based on PRACTALL guidelines and expert opinion.[18] For HOFCs, patients were provided with a scheme containing instructions on how to measure the amounts, and wider dose ranges were accepted to accommodate home settings. For nuts, the protocol included the following steps: 1 mg, 3 mg, 10 mg, 30 mg, 100 mg, 300 mg, 1000 mg and 3000 mg of protein. For fruit and vegetables, the protocol included the following steps: holding ½ teaspoon in the mouth for one minute without chewing or swallowing, followed by gradually consuming increasing amounts with ½ teaspoon, 1 teaspoon, 1/8 portion, 1/4 portion, 1/2 portion, and finally 1 whole portion of the provoked food. For soymilk, the following steps were conducted: 0.1 ml, 0.3 ml, 1 ml, 2.2 ml, 6.5 ml, and 21.7 ml soymilk administered in a mixture, followed by open-label doses of 15 ml, 45 ml, and 135 ml of soymilk. Each challenge ended with 1 normal portion of the provoked food. Every patient performed a challenge for at least one BPFA food. Per OFC or HOFC, a specific scheme was used with raw food, processed food or a combination of both, based on the symptoms. If the patient reported symptoms to processed food, both processed and raw foods were tested. If the patient reported symptoms to raw food without symptoms to processed food, only raw foods were tested.

Patients who performed a HOFC were carefully selected and thoroughly informed. They needed to have clear, non-severe symptoms in their clinical history and not be apprehensive about performing the challenge at home. In contrast, patients who did not meet these criteria underwent an OFC. The challenge was considered positive when objective allergic reactions occurred or when subjective symptoms lasted at least 45 minutes. For HOFC, we instructed patients to stop the HOFC in case the following symptoms occurred: visible swelling lips/tongue/throat/eyelid, tightness of throat, hoarseness, swallowing complaints, runny or congested nose, watery eyes, redness of skin, urticaria, or any gastro-intestinal, respiratory or cardiovascular symptom. An expert panel assessed the challenge outcomes including the degree of severity.

Severity of symptoms

Doctor's diagnosed severity was assessed based on symptoms obtained during clinical history by a food allergy expert and second, based on symptoms observed during challenge. This was classified using the oFASS-5 severity score.[19] The oFASS-5 severity score has five grades, which we categorized as follows: oFASS-5 grade 1 was classified as mild, oFASS-5 grades 2 or 3 as moderate, and oFASS-5 grades 4 or 5 as severe.

Patient-perceived severity was also assessed both during clinical history and challenge. This was measured using a numerical rating scale (NRS), ranging from 0 (no symptoms) to 10 (most severe).

From now on, severity scores will be referred to as oFASS clinical severity and NRS patient-perceived severity.

Safety of home-based oral food challenges

The safety of HOFCs was assessed by the number of severe reactions, number of challenges discontinued per protocol, and use of adrenaline.

Statistical analyses

Patients' characteristics were described for continuous variables using mean and standard deviation (SD) when normally distributed or using median with interquartile range (Q1, Q3) when not normally distributed. Categorical variables were described with frequency and percentage.

Analyses were performed using IBM SPSS Statistics version 29.0.1.

RESULTS

Study Population

In total, 38 patients were included with a mean age of 33 years of whom 60.5% were female. Details of the study population are shown in **Table 1**. The median number of food allergies per patient was 8 with a maximum of 16 and the total FAQLQ-AF score was 3.3, indicating moderate impaired HRQL. Co-allergies are shown in **Table S1A**. The majority has a co-allergy to (other) fruits. Co-allergies to vegetables and legumes were less common. Sensitization profiles and co-sensitization patterns are shown in **Table S2** and **Table S1B**, respectively. Co-sensitization to hazelnut extract and PR-10 components was common, whereas co-sensitization to extracts of soy, carrot, and plum was less frequently observed.

Table 1. Baseline characteristics

Baseline characteristics	Mean (sd)*
Total N (%)	38 (100)
Demographics	
Age in years	33.3 (11.4)
Female sex; N (%)	23 (60.5)
Allergic comorbidities	
Allergic rhinitis; N (%)	38 (100)
Asthma; N (%)	16 (42.1)
Atopic dermatitis; N (%)	21 (55.3)
Number of food allergies	7.6 (4.0)
FAQLQ-AF	
Total score	3.3 (1.3)
Food Allergy related Health	3.6 (1.2)
Emotional impact	3.5 (1.5)
Allergen avoidance & Dietary restrictions	3.5 (1.4)
Risk of accidental exposure	3.1 (1.4)

*Values are in mean (sd) unless mentioned otherwise

More than 10% of BPFA patients reported severe symptoms to birch pollen-related foods in clinical history

In clinical history, the food for which patients most frequently reported symptoms was apple (84.2%), followed by kiwi (68.4%), cherry (63.2%), hazelnut (55.3%) and pear (50.0%) (**Figure 1**). Celery was the least frequently reported, with only 5.3% affected. Regarding food groups, symptoms were most commonly reported for fruit (94.7%), followed by nuts (76.3%), vegetables (52.6%) and legumes (36.8%).

Table 2A. Percentages of food allergy symptoms obtained during clinical history

			Oropharyngeal				Mucosa				Eyes		Nose		Skin				Gastro-intestinal					Respiratory				Cardiovascular		
		N symptoms	itching mouth/lips/ears	feeling swelling mouth/lips/ears	itchy throat	Feeling tightness of throat	swallowing complaints	visible swelling lips	visible swelling tongue	visible swelling throat	conjunctivitis	swelling of eyelids	sneezing	runny/congested nose	itchy skin	erythema	1-10 urticaria	>10 urticaria	nausea	abdominal pain	vomiting	diarrhoea	hoarseness	cough	dyspnoea	wheezing	drop in blood pressure	heart rate increase	fainting/dizziness
Fruit	Apple	32	75.0	46.9	65.6	46.9	31.3	31.3	12.5	9.4	15.6	3.1	15.6	18.8	9.4	6.3			3.1	9.4		6.3	3.1	6.3	6.3	6.3			
	Kiwi	26	73.1	42.3	61.5	30.8	11.5	23.1	11.5	3.8	7.7		19.2	11.5	11.5	3.8			3.8	11.5		7.7	3.8	3.8	11.5	7.7			
	Cherry	24	66.7	54.2	50.0	37.5	8.3	33.3	8.3	4.2	4.2	8.3	12.5	8.3	4.2				4.2	4.2				8.3	8.3	4.2			
	Pear	19	73.7	42.1	63.2	31.6	26.3	15.8	5.3	10.5	10.5		15.8	15.8	21.1				5.3	5.3		5.3			5.3				
	Peach	18	66.7	44.4	61.1	38.9	16.7	27.8	5.6	5.6	16.7		16.7	5.6	16.7				5.6	11.1		5.6							
	Strawberry	17	76.5	35.3	70.6	29.4	5.9	11.8			5.9	5.9	11.8	17.6	17.6	11.8						5.9	5.9		11.8				
	Nectarine	16	68.8	37.5	68.8	25.0	18.8	31.3	6.3	6.3	12.5		12.5	6.3	18.8				6.3	12.5		6.3	6.3		6.3				
	Apricot	13	69.2	53.8	69.2	23.1	15.4	23.1	7.7		23.1		23.1	15.4	30.8	7.7			7.7						7.7				
	Plum	11	81.8	54.5	54.5	54.5	18.2	18.2	9.1	9.1	27.3		27.3	9.1	18.2							9.1		9.1	9.1				
	Hazelnut	21	76.2	42.9	61.9	42.9	33.3	23.8	4.8	9.5	4.8	4.8	9.5	4.8	33.3	9.5	4.8		4.8	4.8	9.5	4.8	4.8	14.3	23.8	9.5			
Nuts	Walnut	18	44.4	11.1	50.0	22.2	11.1	11.1		11.1					11.1	5.6													
	Almond	15	46.7	26.7	60.0	26.7	13.3	20.0	6.7	6.7	6.7		13.3		13.3	6.7						6.7			6.7	6.7			
	Pecan nut	8	50.0		37.5	12.5	12.5								25.0	12.5				12.5		25.0							
	Pistachio nut	5	60.0	40.0											40.0	20.0						20.0							
	Cashew nut	5	40.0		20.0	20.0	20.0				20.0				40.0	20.0						20.0							
	Brazil nut	4	50.0		75.0	50.0	25.0																		25.0				
	Macadamia	4	50.0	25.0		25.0	25.0	25.0	25.0						50.0	25.0													

Table 2A. Percentages of food allergy symptoms obtained during clinical history (*continued*)

			Oropharyngeal					Mucosa			Eyes		Nose	Skin				Gastro-intestinal					Respiratory				Cardiovascular		
		N symptoms	itching mouth/lips/ears	feeling swelling mouth/lips/ears	itchy throat	Feeling tightness of throat	swallowing complaints	visible swelling lips	visible swelling tongue	visible swelling throat	conjunctivitis	swelling of eyelids	sneezing	runny/congested nose	itchy skin	erythema	1-10 urticaria	>10 urticaria	nausea	abdominal pain	vomiting	diarrhoea	hoarseness	cough	dyspnoea	wheezing	drop in blood pressure	heart rate increase	fainting/dizziness
	Tomato	13	69.2	38.5	61.5	30.8		15.4	7.7	15.4			15.4	7.7	15.4	15.4	7.7	7.7	7.7	15.4	7.7	15.4			15.4				
Vegtables	Carrot	10	90.0	50.0	80.0	40.0	20.0	30.0	20.0	10.0	10.0	10.0	30.0	20.0	10.0								10.0	10.0	10.0	10.0			
	Bell pepper	10	60.0	40.0	70.0	30.0		30.0							10.0	10.0				10.0					10.0				
	Celery	2	100.0	50.0	100.0	100.0		50.0	50.0		50.0		50.0																
Legumes	Soy milk	8	62.5	62.5	50.0	25.0	37.5	25.0	12.5		12.5		12.5	12.5	12.5				12.5	25.0		12.5	12.5	12.5	25.0	12.5			
	Peanut	8	50.0	37.5	50.0	50.0	12.5	12.5	12.5	12.5					12.5	12.5				12.5		12.5	12.5		12.5	12.5			

Legenda: green = uncommon, red = often reported

Table 2B. Food allergy symptoms obtained during clinical history with corresponding mean patient-perceived severity scores on the numerical rating scale

			Oropharyngeal				Mucosa				Eyes	Nose		Skin				Gastro-intestinal					Respiratory				Cardiovascular		
		N syptoms	itching mouth/lips/ears	feeling swelling mouth/lips/ears	itchy throat	Feeling tightness of throat	swallowing complaints	visible swelling lips	visible swelling tongue	visible swelling throat	conjunctivitis	swelling of eyelids	sneezing	runny/congested nose	itchy skin	erythema	1-10 urticaria	>10 urticaria	nausea	abdominal pain	vomiting	diarrhoea	hoarseness	cough	dyspnoea	wheezing	drop in blood pressure	heart rate increase	fainting/dizziness
	Mean NRS per symptom		5.1	5.8	5.5	5.3	6.1	6.2	3.8	3.2	5.1	4.5	5.8	6.6	4.8	5.5	8.8	8.0	3.2	3.9	6.1	5.7	7.3	5.4	5.8	4.5			
Fruit	Apple	32	5.9	6.3	6.9	5.2	5.7	6.0	4.8	2.7	6.0	1.0	6.6	6.0	7.0	7.0			2.0	6.7		6.5	10.0	6.5	4.0	1.0			
	Kiwi	26	5.4	5.5	5.3	5.5	6.3	6.0	4.7	8.0	3.5		6.4	4.7	3.7	3.0			2.0	3.7		7.0	8.0	6.0	6.3	5.5			
	Cherry	24	6.0	6.0	6.2	6.1	8.0	4.4	2.0	1.0	6.0	6.0	7.0	8.0	7.0				2.0	1.0				3.5	6.0	4.0			
	Pear	19	5.1	4.5	5.5	4.8	4.0	3.7	2.0	2.5	6.0		5.3	5.7	6.5				3.0	4.0		1.0			6.0				
	Peach	18	5.1	5.6	5.0	4.4	4.0	6.2	3.0	1.0	3.0		6.7	8.0	3.0				3.0	4.0		6.0							
	Strawberry	17	4.0	5.2	4.6	4.8	10.0	7.5			3.0	8.0	5.0	5.3	5.7	6.0						7.0	6.0		4.5				
	Nectarine	16	5.8	5.7	5.6	4.8	4.3	6.2	3.0	1.0	1.5		7.0	8.0	5.3				2.0	3.5		6.0	6.0		6.0				
	Apricot	13	5.3	6.0	5.3	7.7	5.5	7.0	2.0		3.7		4.3	5.0	5.5	8.0			1.0						6.0				
	Plum	11	5.8	4.8	6.7	4.8	5.5	6.5	2.0	1.0	4.0		5.7	8.0	4.0							8.0		4.0	7.0				

Table 2B. Food allergy symptoms obtained during clinical history with corresponding mean patient-perceived severity scores on the numerical rating scale (*continued*)

			Oropharyngeal					Mucosa			Eyes		Nose		Skin			Gastro-intestinal				Respiratory			Cardiovascular
Nuts	Hazelnut	21	5.9	6.8	7.1	7.0	6.6	7.4	7.0	5.0	8.0	3.0	6.5	8.0	5.3	5.5	8.0	8.0	2.0	5.5	5.0	5.0	5.3	4.6	4.0	
	Walnut	18	4.3	7.0	4.9	3.5	5.5	7.0		1.5					3.5	6.0										
	Almond	15	4.6	7.5	5.0	2.5	6.0	7.3	3.0	1.0	6.0		5.0		3.5	6.0					8.0			8.0	8.0	
	Pecan nut	8	3.3		3.0	3.0	1.0								3.5	6.0			4.0		5.0					
	Pistachio nut	5	3.0	1.5											3.5	6.0					6.0					
	Cashew nut	5	4.0		7.0	10.0	10.0				6.0				3.5	6.0					6.0					
	Brazil nut	4	4.5		4.0	4.5	1.0																	5.0		
	Macadamia	4	6.0	10.0		10.0	10.0	6.0	8.0						3.5	6.0										
Vegtables	Tomato	13	3.9	4.6	4.9	5.0		7.0	7.0	4.5			1.5	5.0	3.0	5.0	10.0	8.0	7.0	2.0	7.0	2.5			5.5	
	Carrot	10	4.8	6.2	5.1	5.5	9.5	7.7	6.0	9.0	9.0	9.0	7.7	8.0	7.0								9.0	9.0	9.0	9.0	
	Bell pepper	10	3.8	5.3	5.6	4.7		7.3							1.0	1.0				6.0					8.0	
	Celery	2	3.5	4.0	3.5	2.0		4.0	2.0		2.0		2.0													
Legumes	Soy milk	8	5.4	5.0	5.3	7.5	6.3	4.5	1.0		8.0		2.0	8.0	3.0			4.0	7.0		6.0	7.0	2.0	6.0	7.0	
	Peanut	8	5.0	5.3	4.5	4.8	6.0	4.0	2.0	5.0					5.0	5.0			6.0		6.0	4.0		5.0	4.0	

Symptoms reported per food in < 5 patients are shown in grey. Severity in NRS: green = *mild* to red = *severe*

Evaluating the oFASS clinical severity of all individual foods, 48.1% were classified as mild, 40.4% as moderate, and 11.4% as severe. Severe symptoms were most commonly reported for hazelnut (29%), followed by soy milk (25%), apple (19%), plum (18%), and tomato (15%) (**Figure 1**).

To examine the severity of symptoms in clinical history in more detail, the frequency of each individual symptom was determined (**Table 2A**). Oropharyngeal symptoms were most common, with itching of the mouth/lips/ears (40.4%-100.0%) and throat (37.5%-100.0%) being the most prominent, particularly in response to fruit and vegetables. Other commonly reported symptoms, though less frequent than oropharyngeal symptoms, included visible swelling of the lips (11.1%-50.0%), and sneezing (9.5%-50.0%). Dyspnea was reported up to 25%, and was most commonly related to hazelnut and soy milk. Patients reported urticaria or vomiting less frequently, and no cardiovascular symptoms were reported. Overall, 81.6% of patients reported more than only oropharyngeal symptoms.

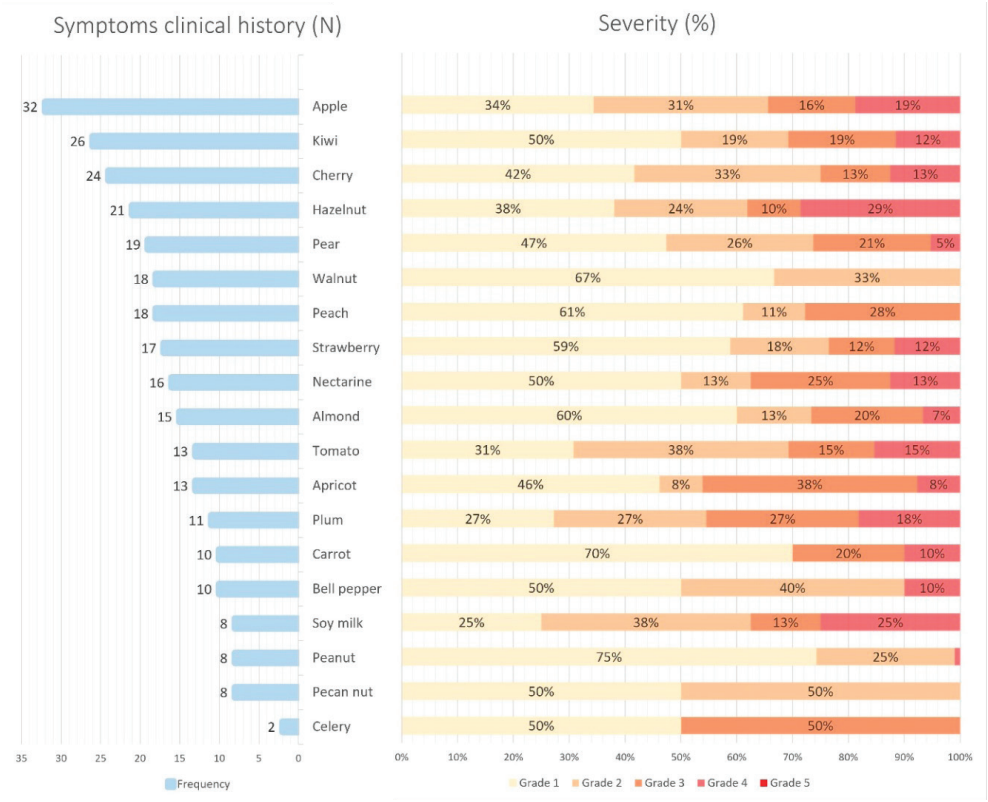


Figure 1. Frequency and severity in oFASS-5 score of food allergy symptoms in clinical history

Regarding the NRS patient-perceived severity, visible swelling of the throat, visible swelling of the tongue, nausea and abdominal pain had the lowest NRS score (between 3.2-3.9) (**Table 2B**). These were followed by symptoms of the eyes, oropharynx, and nose, and most of the respiratory symptoms (NRS between 4.5 – 6.6). Urticaria and hoarseness received the highest NRS score (between 7.3-8.8). Remarkably, symptoms scored as mild by oFASS (i.e., oropharyngeal symptoms) were not perceived by patients with the lowest NRS. Conversely, some symptoms classified as severe by the oFASS (i.e., respiratory symptoms) were not perceived by patients with the highest NRS. This indicates that respiratory symptoms were not at the most extreme end of the severity spectrum.

Clinical severe reactions by oFASS in 12.9% of challenges, but patient perception of these severe symptoms varied considerably

A total of 109 challenges were performed, mostly with a hazelnut product (Nutella, roasted or raw) (50.4%) or with fresh apple (25.7%) (**Table 3**).

Table 3. Overview of oral food challenges and home introduction

Food	Performed N (%)		Pos OFC N (%)		Pos HI N (%)		Subjective median ED	Objective median ED
Total	109	(100)	48/60	(80.0)	45/49	(91.8)	93	30
Apple - fresh	28	(25.7)	6/6	(100)	21/22	(95.5)	0.5 tbsp mouth	1/2 piece
Hazelnut - raw	23	(21.1)	16/17	(94.1)	6/6	(100)	10 mg	30 mg
Hazelnut -roasted	19	(17.4)	11/16	(68.8)	2/3	(66.7)	1000 mg*	30 mg
Hazelnut - Nutella	13	(11.9)	7/11	(63.6)	1/2	(50.0)	10 mg	10 mg
Carrot - fresh	5	(4.6)	1/1	(100)	4/4	(100)	0.5 tbsp mouth	0.5 tbsp eat
Carrot -processed	1	(0.9)	1/1	(100)	0/0		1/8 piece	NA
Walnut - raw	4	(3.7)	1/2	(50)	2/2	(100)	10 mg	100 mg
Walnut -processed	2	(1.8)	0/0	-	1/2	(50.0)	3 mg	NA
Cherry - raw	3	(2.8)	3/3	(100)	0/0	-	0.5 tbsp mouth	1 tbsp eat
Cherry -processed	1	(0.9)	1/1	(100)	0/0	-	0.5 tbsp eat	NA
Kiwi - fresh	3	(2.8)	0/0	-	3/3	(100)	0.5 tbsp mouth	0.5 tbsp mouth
Peach - fresh	3	(2.8)	0/0	-	3/3	(100)	0.5 tbsp mouth	NA
Soymilk	2	(1.8)	1/2	(50)	0/0	-	135 ml	135 ml
Almond - processed	1	(0.9)	0/0	(0)	1/1	(100)	0.5 tbsp mouth	60 gram
Plum - fresh	1	(0.9)	0/0	-	1/1	(100)	0.5 tbsp mouth	NA

*Roasted hazelnut was often challenged together with Nutella. In that case, the first ED of roasted hazelnut was 1000 mg. *Abbreviations: ED = eliciting dose, HI = home introduction, NA = not applicable, OFC = oral food challenge, pos = positive, tbsp mouth = tablespoon held in the mouth for 60 seconds and spat out, tbsp eat = tablespoon consumed*

The NRS patient-perceived mean symptom severity during challenges was 4.4 for oral symptoms, 3.9 for skin and mucous symptoms, 3.6 for gastrointestinal symptoms, and 3.4 for pulmonal symptoms, all indicating moderate impairment. In total, 84.2% of patients experienced more than only oropharyngeal symptoms during challenge. Assessing the oFASS clinical severity of all challenges, 43.0% were classified as mild, 44.1% as moderate, and 12.9% as severe.

Table 4. Detailed overview of all oFASS clinical severe challenges (i.e. at least oFASS-5 score 4)

Food	OFC/ HOFC	Symptoms	Pulmonal NRS	Peak flow	Medication	Subjective threshold	Objective threshold	Stopped before the last dose
Soy milk	OFC	OAS, erythema, abdominal pain, feeling unwell, voice change, feeling throat tightness, dyspnea, coughing, wheezing, sweating	3	550 --> 520 (after medication)	Clemastine 2 mg iv Dexamethason 10 mg iv	135 ml	135 ml	no
Hazelnut - Nutella	OFC	OAS, dyspnea, hoarseness, coughing	5	340 --> 220	Clemastine 2 mg iv	10 mg	10 mg	Yes
Hazelnut - raw	OFC	OAS, sneezing, hoarseness, feverish feeling	6	240 --> 200	No	30 mg	300 mg	no
Hazelnut – Nutella	OFC	OAS, feeling tightness of throat, coughing	2	470 --> 440	No	3 mg	10 mg	no
Hazelnut – roasted	OFC	OAS, itchy skin, dyspnea, hoarseness, coughing, sweating, feeling of panic	4	430 --> 390	No	1000 mg	1000 mg	no
Hazelnut – raw	OFC	OAS, itchy skin, erythema, objective swelling lips, rhinitis, coughing	5	450 --> 450	No	30 mg	30 mg	no
Appel - fresh	OFC	OAS, feeling swelling mouth, conjunctivitis, itchy skin, regurgitation, hoarseness coughing, feverish feeling	7	only baseline	No	1/2 tablespoon mouth	1/2 tablespoon mouth	no
Cherry – fresh	OFC	OAS, mild visible swelling lip and throat, hoarseness	1	only baseline	No	1/2 tablespoon eat	1 tablespoon eat	no

Table 4. Detailed overview of all oFASS clinical severe challenges (i.e. at least oFASS-5 score 4) (*continued*)

Food	OFC/ HOFC	Symptoms	Pulmonal NRS	Peak flow	Medication	Subjective threshold	Objective threshold	Stopped before the last dose
Hazelnut - raw	HOFC	OAS, mild visible swelling mouth/lips, nausea, coughing	unknown	NA	No	3 mg	10 mg	yes
Hazelnut - raw	HOFC	OAS, feeling tightness of throat, swallowing complaints, dyspnea	2	NA	No	3 mg	3 mg	yes
Walnut – raw	HOFC	OAS, feeling swelling mouth/lips/ears, coughing	3	NA	No	3 mg	100 mg	yes
Carrot – fresh	HOFC	OAS, feeling tightness of throat, dyspnea, coughing	5	NA	No	1/2 tablespoon mouth	1/2 tablespoon eat	yes

Abbreviations: HOFC = home-based oral food challenge, OFC = oral food challenge, NRS = numeric rating scale

When examining the oFASS clinical severe symptoms more closely, all patients had pulmonal symptoms (**Table 4**). Two patients (16.7%) received medication for their symptoms, five (41.7%) showed a decrease in peak flow, and in five patients (41.7%) the challenge was discontinued before the final dose. The NRS patient-perceived severity for pulmonal symptoms ranged from 1-7. This suggests that, although all patients exhibited pulmonary symptoms classified as severe according to the oFASS clinical score, the perceived severity varied considerably among individuals.

Hazelnut processing influences challenge outcomes

Among the positive hazelnut challenges, Nutella most frequently resulted in severe symptoms (25.0%) followed by raw (18.0%) and roasted (8%) hazelnut (**Figure 2**). However, not all patients performed challenges for all hazelnut products. Hazelnut products that were tolerated based on clinical history were not challenged. Additionally, if a patient had a severe reaction to processed hazelnut during a challenge, further challenges with less processed forms were not performed. When adjusting for this, raw hazelnut most frequently resulted in severe reactions (16.0%), followed by Nutella (8.0%) and roasted hazelnut (4%)(**Table 5**).

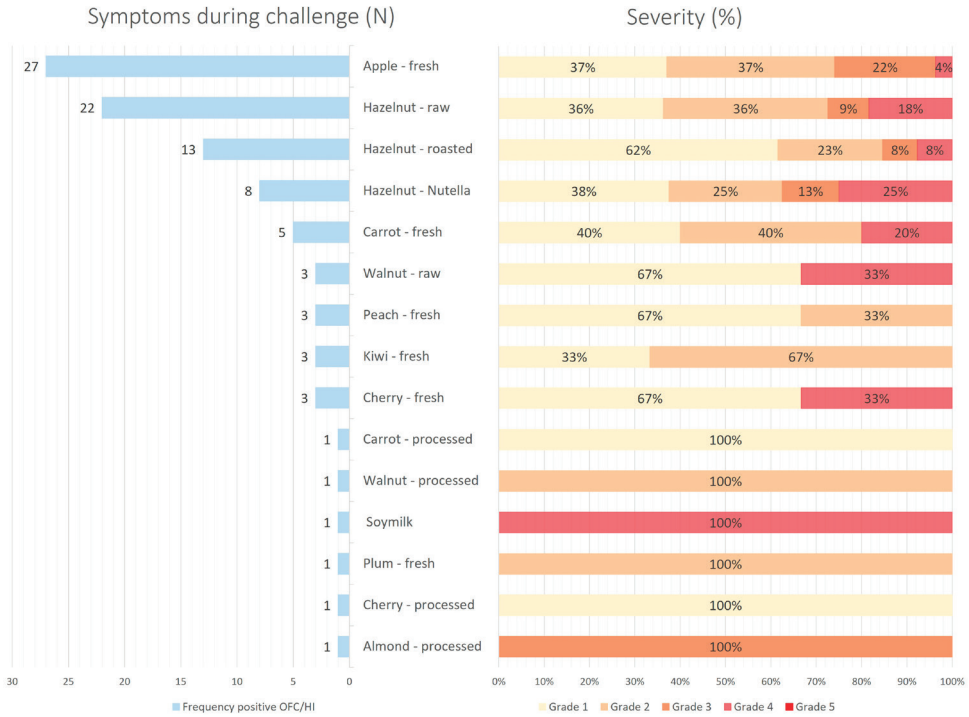


Figure 2. Frequency of positive challenges (oral food challenge or home-based oral food challenge) and severity of symptoms

One patient experienced severe symptoms during the Nutella challenge, no symptoms during the roasted hazelnut challenge and moderate symptoms during the raw hazelnut challenge. This may be partially explained by the fact that the patient also has a known soy allergy, and soy is one of the ingredients in Nutella.

Overall, processing appears to influence the severity of symptoms during challenge, with raw hazelnut causing most frequently severe symptoms, followed by Nutella, and roasted hazelnut.

Table 5. Symptoms severity during challenge per hazelnut product

Hazelnut - raw	Hazelnut - roasted	Hazelnut - Nutella
4	4	1
4	1	1
4	1	1
4	0	0
3	1	1
3	1	0
2	1	2

Table 5. Symptoms severity during challenge per hazelnut product (*continued*)

Hazelnut - raw	Hazelnut - roasted	Hazelnut - Nutella
2	1	0
2	1	0
2	0	4
2	0	0
2	0	0
2	0	0
2	0	0
1	3	0
1	2	2
1	2	0
1	1	1
1	1	0
1	1	0
1	0	0
1	0	0
0	0	0
NP	NP	4
NP	NP	3

Legend: 0 = no symptoms*; 1 = oFASS-5 grade 1, 2 = oFASS-5 grade 2, 3 = oFASS-5 grade 3, 4 = oFASS-5 grade 4, NP = no challenge performed, due to severe reaction to processed Nutella
*Either a negative challenge, or no challenge performed if no symptoms were reported in clinical history

Home introductions are safe when patients are carefully pre-selected, well informed, and guided

Of the 109 challenges, 45.0% were performed at home, with positive outcomes in 91.8% of cases (**Table 3**). Fresh apple (44.9%) was most frequently challenged by HOFC, followed by one of the hazelnut products (22.4%).

Regarding oFASS clinical severity, 33.3% of HOFCs were classified as mild, 57.8% as moderate, and 8.9% as severe. Although patients had severe symptoms, none of them required adrenaline as rescue medication and the patient-perceived severity of these severe symptoms was scored with a mean NRS of 3.2, indicating mild impairment. In total, 55.1% of HOFCs were discontinued per protocol, to reduce the risk of severe symptoms at home.

As none of the patients required adrenaline as rescue medication, and the mean NRS patient-perceived severity of the oFASS clinically severe symptoms was only 3.2, the findings support the safety of HOFC. Furthermore, patients followed the instructions for

stopping the challenge appropriately, demonstrating that HOFC can be safely performed in well-selected low-risk individuals. These results suggest that, with careful patient selection and clear instructions HOFC is safe.

DISCUSSION

This is the first study to provide an in-depth characterization of patients with BPFA confirmed by food challenges and supported by molecular characterization. During challenges, 84.2% of patients experienced more than just oropharyngeal symptoms, and 12.9% of challenges were classified as severe according to the oFASS score. Remarkably, patient's perspective severity of these oFASS severe symptoms varied considerably, with NRS scores indicating moderate impairment. The degree of hazelnut processing was shown to influence allergenicity, with raw being most severe. Finally, in patients at low risk of severe reactions, who receive clear instructions and an emergency action plan, HOFCs were shown to be safe.

Moderate to severe symptoms do occur in patients with BPFA

BPFA is generally mild and often characterized by only local oropharyngeal symptoms.[15,20] Our study confirmed that oropharyngeal symptoms, like itching of the mouth, lips and/or ears and itching of the throat were indeed the most frequently reported symptoms. However, during challenges, we also found that a substantial proportion of patients experienced oFASS clinically moderate (43.0%) and even severe reactions (12.9%), suggesting that BPFA can present with a broader and more serious spectrum of symptoms than previously assumed.[15,20] It is important to note, that the oFASS scoring system does not distinguish between varying degrees of pulmonary symptoms. For example, mild dyspnea and severe respiratory distress has the same severity score. This limitation may overestimate the clinical severity in some cases. Supporting this, the mean NRS patient-perceived severity score for symptoms classified as "severe" by oFASS was 3.4 (with a maximum of 7), indicating that most patients did not perceive their symptoms as being at the most severe end of the spectrum. Collectively, these findings confirm that BPFA, while often mild, can result in severe reactions and should not be underestimated.

Processing decreases allergenicity

The severity of hazelnut challenges is likely related to processing: raw hazelnut caused most frequently severe reaction, followed by Nutella, and roasted hazelnut. Although the exact processing steps of Nutella are proprietary, roasted hazelnuts are generally produced using temperatures between 100–160°C for 10–60 minutes.[21] As roasting reduces the allergenicity of PR-10 proteins in hazelnut[22], it might be that the hazelnuts used in Nutella were roasted at lower temperature or shorter duration than those used in the roasted hazelnuts during the challenge, and therefore elicited more severe symptoms.

Another factor potentially contributing to the unexpectedly high severity of reactions to Nutella may be its high-fat matrix. Nutella contains 13% hazelnut and lists hazelnut as the third ingredient, indicating that the first two ingredients—sugar and palm oil—are present in higher amounts. Studies performed with peanut showed that peanut challenged with a high-fat peanut matrix caused more severe symptoms than with a lower-fat matrix.[23,24]

Home introductions are safe in patients with BPFA

OFCs are commonly used to confirm the presence of a food allergy, determine the patients' threshold dose, and characterize the specific symptoms elicited.[4,5,15] We showed that HOFC can be valuable to confirm an allergy and estimate the threshold dose. However, when it comes to evaluating the full spectrum of symptoms, HOFCs may lead to underrepresentation due to discontinuation per protocol. In our study, the safety of HOFCs was ensured by detailed patient instructions, including clear criteria for when to proceed and when to stop. Any occurrence of objective symptoms prompted immediate discontinuation of the HOFC to prevent potential severe or life-threatening reactions. As a result, the discontinuation rate of HOFCs was relatively high (55.1%) compared to OFCs (8.3%). It is possible that, had the HOFCs not been terminated early, the severity of symptoms would have been greater than observed.

To our knowledge, no other studies have investigated HOFCs. Since this approach could enable faster diagnosis and more targeted dietary advice, we recommend further studies to support our data and incorporation in daily practice. With clear instructions and proper patient selection, low risk patients can safely perform challenges at home without the need to wait for a hospital-based OFC. Early confirmation of whether a patient is truly allergic, and to which forms of the food, allows for timely, individualized dietary guidance. This is crucial to prevent unnecessary food restrictions, reduce the risk of nutritional deficiencies and accidental reactions, to support overall HRQL, and may prevent development of (severe) allergy.[25–27]

Our results suggest that HOFCs can be considered a safe and practical method to assess presence (or absence) and threshold of food allergy in carefully selected and well-informed BPFA patients. It is important to continue investigating HOFCs in other and larger populations as these could not only alleviate the pressure and costs on our healthcare system but also offer patients quicker access to tailored dietary advice.

Strengths and limitations

The primary limitation of our study is the relatively small sample size of 38 patients. Despite this, BPFA was based on challenges for at least one BPFA food, resulting in a total of 109 challenges, which allowed for a detailed and robust assessment of symptom severity. In addition, we comprehensively addressed reactivity to 19 different foods and

captured NRS scores for 27 distinct symptoms, complemented by molecular characterization. Another limitation is that PR-10 and non-PR-10 components are not available for all BPFA-related foods. However, we included the most relevant components, and, to our knowledge, this is the first study to incorporate such detailed molecular characterization of BPFA.

CONCLUSIONS

BPFA is mild in most patients, but over 80% experience symptoms beyond the oropharynx, and even 12.9% have severe reactions. HOFCs are safe for well-selected patients with clear guidance, offering a promising way to reduce hospital resource use and improve allergy management.

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SUPPLEMENTAL

Table S1A. Co-allergy of self-reported symptoms of birch pollen-related foods

When allergic to …		% of patients also allergic to																			
		Fruit									*Nuts*				*Vegtables*				*Legumes*		
	N	Apple	Kiwi	Cherry	Pear	Peach	Strawberry	Nectarine	Apricot	Plum	Hazelnut	Walnut	Almond	Pecan nut	Tomato	Bell pepper	Carrot	Celery	Peanut	Soy milk
Fruit Apple	32	100	71.9	71.9	59.4	53.1	50.0	46.9	40.6	34.4	53.1	50.0	43.8	21.9	37.5	31.3	25.0	6.3	21.9	21.9
Fruit Kiwi	26	88.5	100	57.7	65.4	57.7	61.5	57.7	42.3	30.8	57.7	53.8	38.5	23.1	50.0	34.6	38.5	7.7	19.2	19.2
Fruit Cherry	24	95.8	62.5	100	58.3	62.5	50.0	50.0	41.7	45.8	58.3	41.7	41.7	20.8	33.3	29.2	20.8	8.3	25.0	29.2
Fruit Pear	19	100	89.5	73.7	100	68.4	63.2	57.9	63.2	52.6	57.9	63.2	36.8	26.3	57.9	42.1	36.8	10.5	21.1	26.3
Fruit Peach	18	94.4	83.3	83.3	72.2	100	55.6	77.8	55.6	50.0	61.1	50.0	33.3	16.7	44.4	33.3	33.3	5.6	16.7	27.8
Fruit Strawberry	17	94.1	94.1	70.6	70.6	58.8	100	58.8	47.1	35.3	52.9	52.9	47.1	23.5	47.1	52.9	52.9	11.8	29.4	23.5
Fruit Nectarine	16	93.8	93.8	75.0	68.8	87.5	62.5	100	68.8	50.0	81.3	56.3	43.8	25.0	56.3	37.5	37.5	6.3	25.0	31.3
Fruit Apricot	13	100	84.6	76.9	92.3	76.9	61.5	84.6	100	61.5	69.2	61.5	38.5	30.8	46.2	38.5	30.8	7.7	23.1	30.8
Fruit Plum	11	100	72.7	100.0	90.9	81.8	54.5	72.7	72.7	100	72.7	45.5	27.3	27.3	63.6	36.4	27.3	9.1	27.3	36.4
Nuts Hazelnut	21	81.0	71.4	66.7	52.4	52.4	42.9	61.9	42.9	38.1	100	66.7	52.4	33.3	33.3	14.3	28.6	9.5	23.8	33.3
Nuts Walnut	18	88.9	77.8	55.6	66.7	50.0	50.0	50.0	44.4	27.8	77.8	100	50.0	38.9	33.3	27.8	38.9	5.6	27.8	16.7
Nuts Almond	15	93.3	66.7	66.7	46.7	40.0	53.3	46.7	33.3	20.0	73.3	60.0	100	46.7	40.0	33.3	26.7	13.3	26.7	26.7
Nuts Pecan nut	8	87.5	75.0	62.5	62.5	37.5	50.0	50.0	50.0	37.5	87.5	87.5	87.5	100	25.0	25.0	25.0	12.5	25.0	12.5
Vegtables Tomato	13	92.3	100	61.5	84.6	61.5	61.5	69.2	46.2	53.8	53.8	46.2	46.2	15.4	100	53.8	30.8	7.7	23.1	23.1
Vegtables Bell pepper	10	100	90.0	70.0	80.0	60.0	90.0	60.0	50.0	40.0	30.0	50.0	50.0	20.0	70.0	100	60.0	10.0	20.0	20.0
Vegtables Carrot	10	80.0	100	50.0	70.0	60.0	90.0	60.0	40.0	30.0	60.0	70.0	40.0	20.0	40.0	60.0	100	20.0	20.0	20.0
Vegtables *Celery*	*2*	*100*	*100*	*100*	*100*	*50.0*	*100.0*	*50.0*	*50.0*	*50.0*	*100*	*50.0*	*100*	*50.0*	*50.0*	*50.0*	*100*	*100*	*0.0*	*50.0*
Legumes Peanut	8	87.5	62.5	75.0	50.0	37.5	62.5	50.0	37.5	37.5	62.5	62.5	50.0	25.0	37.5	25.0	25.0	0.0	100	25.0
Legumes Soy milk	8	87.5	62.5	87.5	62.5	62.5	50.0	62.5	50.0	50.0	87.5	37.5	50.0	12.5	37.5	25.0	25.0	12.5	25.0	100

Table S1B. Co-sensitization of ImmunoCAP measures of birch pollen-related foods

When sensitized to …		% of patients also sensitized to																	
s IgE to	**N sensitized**	**Bet v 1**	**Birch**	**Hazel-nut**	**Pru p 1**	**Cor a 1**	**Mal d 1**	**Act d 8**	**Ara h 8**	**Peach**	**Gly m 4**	**Ap-ple**	**Api g 1**	**Cel-ery**	**Pea-nut**	**Kiwi**	**Cher-ry**	**Soy**	**Car-rot**	**Plum**
Bet v 1	38	100	100	97.4	94.7	94.7	92.1	89.5	89.5	78.9	78.9	65.8	65.8	63.2	47.4	44.7	28.9	15.8	13.2	5.3
Birch	38	100	100	97.4	94.7	94.7	92.1	89.5	89.5	78.9	78.9	65.8	65.8	63.2	47.4	44.7	28.9	15.8	13.2	5.3
Hazelnut	37	100	100	100	94.6	97.3	91.9	89.2	89.2	78.4	78.4	64.9	67.6	64.9	45.9	43.2	29.7	13.5	13.5	5.4
Pru p1	36	100	100	97.2	100	94.4	94.4	91.7	91.7	83.3	80.6	66.7	66.7	63.9	47.2	47.2	30.6	16.7	13.9	5.6
Cor a1	36	100	100	100	94.4	100	91.7	88.9	88.9	77.8	80.6	66.7	69.4	66.7	47.2	44.4	30.6	13.9	13.9	5.6
Mal d1	35	100	100	97.1	97.1	94.3	100	91.4	94.3	85.7	82.9	71.4	68.6	68.6	51.4	48.6	31.4	17.1	14.3	5.7
Act d 8	34	100	100	97.1	97.1	94.1	94.1	100	94.1	85.3	82.4	70.6	73.5	70.6	52.9	50.0	32.4	17.6	14.7	5.9
Ara h 8	34	100	100	97.1	97.1	94.1	97.1	94.1	100	85.3	88.2	70.6	73.5	67.6	52.9	50.0	32.4	17.6	11.8	5.9
Peach	30	100	100	96.7	100	93.3	100	96.7	96.7	100	83.3	73.3	70.0	70.0	50.0	53.3	33.3	20.0	16.7	6.7
Gly m 4	30	100	100	96.7	96.7	96.7	96.7	93.3	100	83.3	100	76.7	80.0	70.0	56.7	56.7	33.3	20.0	13.3	6.7
Apple	25	100	100	96.0	96.0	96.0	100	96.0	96.0	88.0	92.0	100	72.0	76.0	72.0	64.0	40.0	24.0	16.0	8.0
Api g 1	25	100	100	100	96.0	100	96.0	100	100	84.0	96.0	72.0	100	84.0	56.0	56.0	40.0	16.0	16.0	8.0
Celery	24	100	100	100	95.8	100	100	100	95.8	87.5	87.5	79.2	87.5	100	66.7	58.3	37.5	20.8	20.8	8.3
Peanut	18	100	100	94.4	94.4	94.4	100	100	100	83.3	94.4	100	77.8	88.9	100	66.7	38.9	33.3	16.7	5.6
Kiwi	17	100	100	94.1	100	94.1	100	100	100	94.1	100	94.1	82.4	82.4	70.6	100	41.2	35.3	11.8	11.8
Cherry	11	100	100	100	100	100	100	100	100	90.9	90.9	90.9	90.9	81.8	63.6	63.6	100	9.1	18.2	9.1
Soy	6	100	100	83.3	100	83.3	100	100	100	100	100	100	66.7	83.3	100	100	16.7	100	16.7	16.7
Carrot	5	100	100	100	100	100	100	100	80.0	100	80.0	80.0	80.0	100	60.0	40.0	40.0	20.0	100	20.0
Plum	2	100	100	100	100	100	100	100	100	100	100	100	100	100	50.0	100	50.0	50.0	50.0	100

Table S2. Sensitization profiles measured by ImmunoCAP, immunoblot skin prick test or prick to prick test

	ImmunoCAP (kU_A/L)			Immunoblot (classes)			SPT / PTP (mm)		
	N	Mean	SD	N	Median	IQR	N	Median	IQR
Total IgE	38	287.34	322.60						
Birch	38	35.06	34.79	38	62.0	34.5	38	7.5	3.125
Bet v 1	38	34.97	35.46						
Apple	38	2.58	6.29	38	14.0	32.5	38	6.5	5.875
Mal d 1	38	15.42	22.82						
Apricot	2	0.25	0.35						
Hazelnut	37	17.17	22.83	38	8.5	17.0	38	5.75	3.5
Cor a 1	36	25.12	31.04	38	70.5	53.3			
Cor a 14	37	0.02	0.05	38	1.0	1.0			
Cor a 9	37	0.02	0.02	38	1.0	0.0			
Carrot	6	2.08	1.85				37	3	4.5
Walnut	4	0.42	0.50	38	1.0	1.3	38	2	3
Cherry	12	3.53	3.99				3	6	
Kiwi	37	1.10	2.18	38	2.0	1.0	37	4.5	3.25
Act d 8	37	8.45	11.34						
Peach	34	5.72	10.20	38	5.0	15.8	37	4	4.5
Pru p 1	37	16.68	2.31						
Pru p 3	34	0.04	0.05						
Soy	38	0.21	0.43	38	0.0	2.0	38	0	3
Gly m 4	38	7.67	10.91						
Gly m 5	38	0.00	0.00						
Gly m 6	38	0.00	0.00						
Soy milk							11	6	5
Tofu							10	3	4.125
Tempeh							10	2	2.25
Almond	8	1.03	1.02	38	2.0	1.3	37	4.5	2.75
Bell pepper							37	2	4
Celery	37	2.01	4.43	38	3.0	2.3	37	0	2.5
Api g 1	36	2.43	2.99						
Pecan				38	2.0	2.75	38	0	0
Peanut	38	1.44	3.21	38	2.0	2.3	37	3	2.75
Ara h 2	38	0.06	0.32	38	1.0	1.0			
Ara h 6	38	0.12	0.73						
Ara h 8	38	10.00	12.00						

Table S2. Sensitization profiles measured by ImmunoCAP, immunoblot skin prick test or prick to prick test (*continued*)

	ImmunoCAP (kU_A/L)			Immunoblot (classes)			SPT / PTP (mm)		
Plum	4	0.32	0.37						
Tomato	1	0.08	-				37	3.5	4.5
PR 10 mix*				38	108.0	60.3			
LTP mix*				38	1.0	1.0			
Nut storage mix*				38	1.0	1.0			
Peanut storage mix*				38	1.0	1.0			

*The mixes included the following allergens: PR-10 mix = Bet v 1, Que a 1, Mal d 1, Jug r 5, Ara h 8; LTP mix = Pru p 3, Tri a 14, Pha v 3, Lac s 1, Mal d 3, Jug r 3, Art v 3, Cor a 8, Ara h 9; nut storage mix = Ana o 3, Jug r 1, Ber e 1; peanut storage mix = Ara h 1, 2, 3, 6, 7

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The effect of subcutaneous and sublingual birch pollen immunotherapy on birch pollen-related food allergy: a systematic review

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Background

Birch pollen-related food allergy (BPFA) is the most common food allergy in birch endemic areas such as Western and Central Europe. Currently, there is no treatment available for BPFA. Due to the cross-reactivity between birch pollen and a range of implicated plant foods, birch pollen allergen immunotherapy (AIT) might be effective in the treatment of BPFA. In this study, we systematically evaluate the effectiveness of birch pollen–specific subcutaneous or sublingual immunotherapy in treating BPFA.

Methods

A search was performed in the PubMed, Embase and Cochrane libraries. Studies were independently screened by two reviewers against predefined eligibility criteria. The outcomes of interest were change in 1) severity of symptoms during food challenge, 2) eliciting dose (ED) and/or 3) food allergy-related quality of life (FA-QoL). The validity of the selected articles was assessed using the Revised Cochrane risk of bias tool. We focused on studies with the lowest risk of bias and considered studies with a high risk of bias as supportive. Data were descriptively summarized.

Results

Ten studies were selected that included 475 patients in total. Seven studies were categorized into "high risk of bias" and three into "moderate risk of bias." The three moderate risk of bias studies, with a total of 98 patients, reported on severity of symptoms during challenge and on the ED. All three studies had a control group. Compared to the control group, improvement in severity of symptoms was observed during challenge in two out of the three studies and on the eliciting dose in one out of three. Only one study investigated the effect of birch pollen AIT on FA-QoL, showing that there was no significant difference between patients receiving subcutaneous immunotherapy or a placebo. Of the seven supportive studies, four had a control group and of those, three showed improvement on both severity of symptoms and ED. None of the supportive studies investigated the effect of the therapy on FA-QoL.

Conclusion

This systematic review shows that there is not enough evidence to draw firm conclusions about the effect of AIT on BPFA. Future research is warranted using robust clinical studies that include long-term effects, QoL, and multiple BPFA-related foods.

INTRODUCTION

In Europe, the rate of prevalence of birch pollen sensitization ranges from approximately from 8% to 16% and climate change is likely to cause this number to increase over time.[1,2] Pollen derived from the *Betulaceae* and *Fagaceae* family constitutes the most prominent source of tree pollen in the Western and Central part of Europe.[2] The major birch pollen allergen, Bet v 1, is a PR-10 protein whose homologous structures are present in a large number of plant foods.[3] Due to cross-reactivity between Bet v 1 and these homologues in foods, approximately 70% of birch pollen-allergic patients reports allergic reactions to foods, commonly referred to as birch pollen-related food allergy (BPFA).[3] BPFA is the most common type of food allergy in Western and Central Europe involving many different foods and food groups, for example, Rosaceae fruits such as apples and peaches, tree nuts such as hazelnuts and walnuts, and vegetables such as carrots, celeriac, and soy. [2,4]

Symptoms of BPFA are usually mild and restricted to the oral cavity; hence, they are often referred to as oral allergy syndrome (OAS). However, more severe allergic reactions with cardiovascular symptoms or even anaphylaxis can occur and are described for e.g., soy protein containing food.[2,5]

To date, no treatment is available for BPFA. The evidence for the effectiveness of oral immunotherapy to foods of BPFA is sparse.[6] Because birch pollen is the primary sensitizer in BPFA, birch pollen AIT has often been considered possibly effective also in the treatment of BPFA.[3] However, there is no evidence supporting this and it is even hypothesized that due to insufficient homology between Bet v 1 and plant food allergens it is not possible to alleviate BPFA symptoms.[7] Both subcutaneous (SCIT) and sublingual (SLIT) immunotherapy are available for birch pollen allergy, but their effectiveness for treating associated food allergies remains a matter of debate.[8,9]

The aim of this review was to systematically evaluate the effect of birch pollen–specific SCIT and SLIT on BPFA with regard to severity of symptoms during challenge, eliciting dose (ED), and food allergy-related quality of life (FA-QoL).

METHODS

Eligibility criteria, information sources, and search

A systemic search strategy (**Supplemental 1**) was developed by combining synonyms for the patient population and intervention using both keywords and medical subject headings. The patient population exists of patients with birch pollen *and* birch pollen-related food allergy; the intervention exists of subcutaneous *or* sublingual birch pollen-specific immunotherapy. Our search was performed in the PubMed, Embase, and Cochrane libraries on 3 November 2022.

Study selection

The citations from PubMed, Embase and the Cochrane library were imported into the Rayyan tool for removing duplicates and for screening. Two authors (J.L. and E.K.) independently screened the titles and abstracts. When a paper was deemed possibly relevant, the full text was also independently screened by these two authors. Selection was based on consensus, and discrepancies were resolved by two other authors (T.L. and P.W.). Included were English language articles that met the following criteria: (1) subjects with a birch pollen allergy, (2) subjects with BPFA for at least one food, (3) those with either birch pollen-specific SCIT or birch pollen-specific SLIT as an intervention, and (4) studies in which the effectiveness of this treatment was evaluated by food challenge. Excluded were studies focusing on food allergy immunotherapy, non-original studies (editorials and expert opinion), conference abstracts, case studies, and animal studies. Reviews were also excluded, but were used to obtain additional articles of interest based on reference checking.

Date extraction

Two authors (J.L. and E.K.) independently recorded the characteristics of the selected studies using a predefined checklist, compromising the following items: 1) study information (first author, year of publication, and country in which the study was performed); 2) study design (randomized controlled trial [RCT]) or comparative/single-arm prospective cohort); 3) type of food challenge (double-blind, placebo-controlled food challenge [DBPCFC] or open food challenge [OFC]); 4) type of food; 5) treatment group characteristics (number of patients and type of immunotherapy), if applicable; 6) control group characteristics (number of patients and type of control); 7) timepoint when outcome was measured; and 8) type of reported outcomes. The extracted outcome measurements were change in 1) severity of symptoms during challenge, 2) eliciting dose and/or 3) food allergy related quality of life. Improvement in the eliciting dose was defined as the percentage of patients who could tolerate at least one higher dose without symptoms during the last performed food challenge compared with baseline.

Risk of bias assessment

The validity of the selected studies was assessed using the Revised Cochrane risk of bias tool (RoB2)[10], which evaluated five domains of bias: D1, the randomization process; D2, deviations from intended interventions; D3, missing outcome data; D4, measurement of the outcome; and D5, selection of the reported result. The following information was assessed; D1, performance of randomization, observed baseline differences in patient characteristics, and concealment of allocation sequence; D2, awareness of the assigned intervention, deviations from the intended intervention due to the trial context, and whether the analysis used to estimate the effect of assignment to the intervention was appropriate; D3, availability of outcome data; D4, whether the method of measuring the outcome was appropriate, comparable between intervention groups, and insensitive to awareness of received intervention; and D5, whether data analysis was prespecified. Each of the questions in the domains could be answered with 'yes', 'probably yes', 'probably no', 'no' and 'no information', which led to a risk of bias per domain classified as 'low', 'some concerns', or 'high'. Furthermore, the overall risk of bias was determined. Single arm studies scored high for domains 1 and 2 because there was no control group/randomization (treatment effect estimates concerned pre-/post-treatment differences) and patients and caregivers were aware of the received intervention.

Synthesis of results

Due to evident heterogeneity between the studies in terms of design, timepoint when outcome was measured, type of food, type of immunotherapy, type of control group, and availability and measurement of the five outcomes, it was considered inappropriate to pool results. Therefore, a qualitative synthesis of the available results was performed without producing a formal statistical summary. Studies with the lowest risk of bias with a control group were considered the most important, while studies with the highest risk of bias without a control groups were considered only supportive. Furthermore, a distinction was made between the direction of effect (positive, no effect, and negative) and the size of the effect in case of an effect (large or small). Based on clinical interpretation, the size of effect was considered large when there was an improvement of at least 20%. In the summary of the effect of birch pollen AIT on BPFA, only objective results are shown when a study reported both on subjective and objective results, because these are more reliable.

RESULTS

Study selection

Our search yielded 3652 unique articles (**Figure 1**). After screening the articles by title, abstract, full text, and reference checking, 10 articles were included.

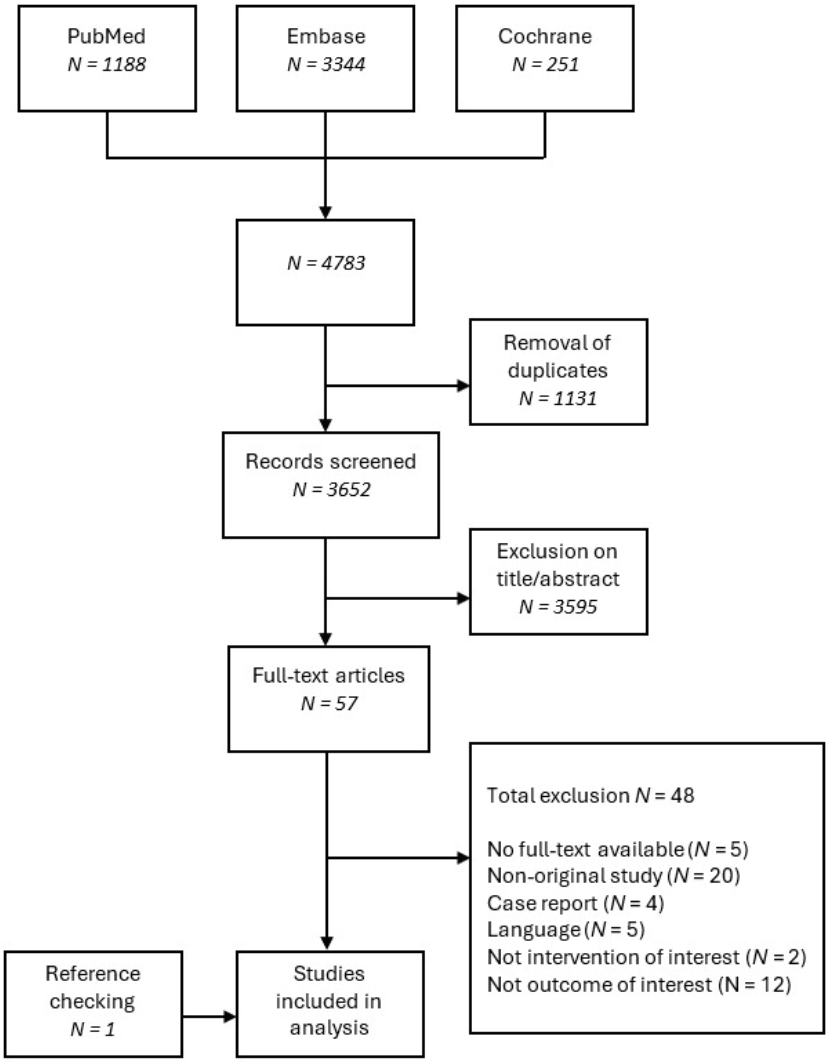


Figure 1. Flowchart of literature search in PubMed, Embase and Cochrane

Study characteristics

Details of the 10 selected studies can be found in **Table 1**. All studies were conducted in Europe. In total, there were five RCTs (of which one was a sub-study of an RCT) and five prospective cohort studies. A control group was used in all RCTs and in two prospective cohorts. In four out of five RCTs, the control group was a placebo group. In the other studies, the control group consisted of patients without AIT. In the studies without a control group, a pre-/post- AIT comparison of the outcome(s) was made.

Table 1. Characteristics of the included articles

A	Study information (first author, year, country)	Study design	Type of challenge	Type of food	Treatment group characteristics	Control group characteristics	Time measured outcome (months)
1	Treudler, 2017[11], Germany and Switzerland	RCT	DBPCFC	Soya	N = 38 SCIT (Allergopharma) Bet v 1	N = 18 Placebo	12
2	Van Hoffen, 2011[12], The Netherlands	RCT	DBPCFC	Hazelnut	N = 10 SCIT (Alutard SQ) Birch-pollen extract	N = 9 Placebo	12
3	Bolhaar, 2004[13] ,The Netherlands	RCT	DBPCFC	Apple (Golden Delicious)	N = 13 SCIT (Alutard SQ) Birch-pollen extract	N = 10 Without AIT	12
4	Hansen, 2004[14], Denmark	RCT	OFC	Apple (Golden Delicious)	N = 28 - SCIT: N=16 (Phostal) - SLIT: N=12 (Staloral) Both Bet v 1	N = 14 Placebo	24
5	Till, 2020[15,] United Kingdom	RCT (sub study)	OFC	Apple (Golden Delicious)	N = 61 SLIT (12 SQ-Bet, ALK) Birch pollen extract	N = 63 Placebo	6.5-9.5
6	Asero, 1998[16], Italy	Prospective cohort	OFC	Apple (Golden Delicious)	N = 49 SCIT (Allergopharma retard (N=33) or Bayer Alhydrox (N=16)) Both birch-pollen extract	N = 26 Without AIT	12-48
7	Bucher, 2004[17], Switzerland	Prospective cohort	OFC	Apple (Golden Delicious) and hazelnut	N = 15 SCIT (Alutard SQ) Birch-hazel-alder pollen extract (N=9), ash pollen (N=3), grass/rye/birch pollen extract (N=2), birch pollen extract (N=1)	N = 12 Without AIT	12
8	Van der Valk, 2020[18], The Netherlands	Prospective cohort	OFC	Apple (not specified)	N = 5 SCIT (Alutard SQ) Tree mix	-	24
9	Bergmann, 2008[19], Germany	Prospective cohort	OFC	175 foods that induced BPFA symptoms (mainly apple and hazelnut)	N = 102 SLIT (B.U. Pangramin (N=81)) Tree mix	-	12

Table 1. Characteristics of the included articles (*continued*)

A	Study information (first author, year, country)	Study design	Type of challenge	Type of food	Treatment group characteristics	Control group characteristics	Time measured outcome (months)
10	Kinaciyan[20], 2007, Austria	Prospective cohort	OFC and DBPCFC	Apple (Golden Delicious)	N = 20 SLIT (B.U. Pangramin) Bet v 1	-	12

Abbreviations: A = article number, AIT = Allergen Immunotherapy, BPFA = Birch pollen food allergy, DBPCFC = Double-blind, placebo-controlled food challenge, N = Number, OFC = Open food challenge, RCT = Randomized controlled trial, SCIT = Subcutaneous immunotherapy, SLIT = Sublingual immunotherapy

Altogether, 475 patients were analyzed, of whom 320 received AIT and 152 did not. Of the 320 patients that received AIT, 127 served as their own controls. Six studies focused on SCIT, three on SLIT and one on either SCIT or SLIT. The last study was a three-arm study, in which they compared patients receiving SCIT or SLIT or placebo. An OFC was performed in six studies, a DBPCFC in three studies, and both OFC and DBPCFC in one study.

During treatment, outcomes were reported at timepoints between 6.5 and 48 months after the start of the treatment, but in 6 out of 10 studies, they were reported at 12 months.

Risk of bias

The overall risk of bias was moderate in three studies and high in seven. Therefore we focused on the three studies with the lowest risk of bias; the remaining studies were considered to be supportive. The overall risk of bias was mainly high because of issues with domain 2, 'Deviations from the intended interventions', which were attributed to no correction of prognostic factors in non-randomized studies. Details of the assessment per outcome reported are presented in **Table 2**.

Table 2. Risk of bias assessment*

A	Comparator	Outcome	D1	D2	D3	D4	D5	Overall
1	Placebo	Severity of symptoms during challenge	🟢	🟢	🟠	🟢	🟢	🟠
1	Placebo	Eliciting dose	🟢	🟢	🟠	🟢	🟢	🟠
1	Placebo	Food allergy related quality of life	🟢	🟢	🟠	🟢	🟢	🟠
2	Placebo	Severity of symptoms during challenge	🟢	🟠	🟢	🟢	🟠	🟠
2	Placebo	Eliciting dose	🟢	🟠	🟢	🟢	🟠	🟠
3	Control	Severity of symptoms during challenge	🟠	🟠	🟢	🟠	🟠	🟠
3	Control	Eliciting dose	🟠	🟠	🟢	🟠	🟠	🟠
4	Placebo	Severity of symptoms during challenge	🟢	🟢	🔴	🟢	🟢	🔴
4	Placebo	Eliciting dose	🟢	🟢	🔴	🟢	🟢	🔴
5	Placebo	Severity of symptoms during challenge	🟠	🔴	🟢	🔴	🟠	🔴
5	Placebo	Eliciting dose	🟠	🔴	🟢	🔴	🟠	🔴

Table 2. Risk of bias assessment* (*continued*)

A	Comparator	Outcome	D1	D2	D3	D4	D5	Overall
6	Control	Severity of symptoms during challenge	🟠	🔴	🟢	🔴	🟠	🔴
6	Control	Eliciting dose	🟠	🔴	🟢	🔴	🟠	🔴
7	Control	Severity of symptoms during challenge	🔴	🔴	🟢	🔴	🟠	🔴
7	Control	Eliciting dose	🟠	🔴	🟢	🟠	🟠	🔴
8	Baseline	Eliciting dose	🔴	🔴	🟠	🔴	🟠	🔴
9	Baseline	Severity of symptoms during challenge	🔴	🔴	🔴	🔴	🟠	🔴
9	Baseline	Eliciting dose	🔴	🔴	🔴	🔴	🟠	🔴
10	Baseline	Severity of symptoms during challenge	🔴	🔴	🟠	🔴	🟠	🔴

*The Revised Cochrane risk of bias tool (RoB2) was used for the risk of bias assessment[10]
Abbreviations: *A = Article number, D1 = Randomization process, D2 = Deviations from the intended interventions, D3 = Missing outcome data, D4 = Measurement of the outcome, D5 = Selection of the reported result*
🟢 low risk of bias; 🟠 moderate risk of bias; 🔴 high risk of bias

Study outcomes

A summary of the study results regarding the outcomes severity of symptoms during challenge and ED is provided in **Tables 3** and **4**. In both tables, a distinction is made between studies with the lowest and highest risk of bias, and those with or without a control group. In studies with a control group, treatment effects concern the comparison between treatment and control groups, and in studies without a control group, treatment effects concern changes from baseline. Henceforward, for the three studies with the lowest risk of bias, only the comparison between treatment and control group is discussed subsequently. Within these studies, a total of 61 patients received AIT and 37 did not.

Birch pollen AIT seems to have a positive effect on severity of symptoms during challenge

Of the three studies with a moderate risk of bias, two showed a positive effect[11,13] on severity of symptoms and one no effect[12] (**Table 3 and 4**). The first study distinguished between objective and subjective symptoms and showed a decrease in both objective and subjective symptoms in both the treatment and the control group. Although there was not a significant difference in the decrease, the patients in the treatment group tended to have a higher numerical decrease in objective symptoms than those in the control group.[11] The second study with a positive effect showed an improvement of symptoms of 69% in the treatment group versus 0% in the control group.[13]

The study with no effect only mentioned that there was no significant difference in symptoms during challenge; no numbers were mentioned.[12]

The effect of birch pollen AIT on eliciting dose is unclear

Of the three studies with a moderate risk of bias, one showed a positive effect[13], one no effect[11] and one a negative effect[12] on ED (**Table 3 and 4**). The study with a positive effect showed a 24-fold increase in eliciting dose in the treatment group versus no change in the control group. Furthermore, 23% of the patients in the treatment group versus 0% of the patients in the control group reached ingestion of the highest dose without symptoms during the last challenge.[13] The study with a negative effect showed a baseline mean ED that did not change in the treatment group but increased from 0.10 to 1.00 gram in the control group.[12]

Further research is needed to assess the effect of birch pollen AIT on FA-QoL

Only one study with a moderate risk of bias investigated the effect of birch pollen AIT on FA-QoL.[11] This study used the validated Food Allergy Quality of Life Questionnaire - Adult Form (FAQLQ-AF) and showed that there was no significant difference between patients receiving SCIT or a placebo. No studies with a high risk of bias investigated the effect of birch pollen AIT on FA-QoL.

Table 3. Overview of the effect of birch pollen AIT on severity of symptoms during challenge and eliciting dose

A	Change in severity of symptoms during challenge	Change in eliciting dose (gram)
	Comparative studies: numbers are treatment versus control unless mentioned otherwise	
	Studies with a moderate risk of bias	
1	- Subjective symptoms from 95% to 67% vs 83% to 60% - Objective symptoms from 82% to 24% vs 78% to 47% - No significant difference	- Mean ED subjective symptoms from 2.2 to 4.7 vs from 0.7 to 2.2 - Mean ED objective symptoms from 4.7 to 24.7 vs from 2.2 to 24.7 - No significant difference
2	- Numbers not mentioned - No significant difference	- Mean ED from 0.65 to 0.65 vs from 0.10 to 1.00 - No significant difference - Median ED - Higher in 40% vs 67% - Unchanged in 20% vs 11% - Lower in 40% vs 22% - No significant difference, p-value not specified
3	- Improvement: 69% vs 0% - Unchanged: 31% vs 90% - No performed DBPCFC: 0% vs 10%	- Increased by factor 24 vs no change - HD: 23% vs 0%

Table 3. Overview of the effect of birch pollen AIT on severity of symptoms during challenge and eliciting dose (*continued*)

A	Change in severity of symptoms during challenge	Change in eliciting dose (gram)
	Studies with a high risk of bias	
4	SCIT vs SLIT vs placebo - Improvement in mean symptom score (severity 0-3) from 1.8 to 1.2 vs 1.5 to 1.2 vs 2.0 to 1.5 (only in placebo group significant improvement)	SCIT (N= 10) vs SLIT (N=4) vs placebo (N=10) - HD: 10% vs 0% vs 20%
5	- More patients with lower VAS score at each dose than in control group *No OFC at baseline*	- HD: 75% vs 68% *No OFC at baseline*
6	- Improvement in 84% vs 0% - P < 0.001 *Treatment group used OFC; control group used questionnaire*	- HD: 45% vs 0% *Treatment group used OFC; control group used questionnaire*
7	- Subjective symptoms from 60% to 67% vs 83% to 92% - Objective symptoms from 20% to 0% vs 0% to 42%	- Mean ED for objective signs from 12.6 to 32.6 vs 9.8 to 8.5 - HD: 13% vs 0% - Percentage of patients with changed ED - Higher: 87% vs 8% - Unchanged:13% vs 0% - Lower: 0% vs 92% - P < 0.01
	Singe – arm studies: numbers are endpoint versus baseline unless otherwise mentioned	
	Studies with a high risk of bias	
8		- Change in median ED (steps): from 1.4 to 6.0 - HD: 40% vs 0% - Percentage of patients with changed ED: - Higher: 80% - Unchanged: 20%
9	- > 50% improvement in 77% - P < 0.0001 *At baseline questionnaire and at endpoint OFC*	Number of tolerated ingested food types from standard list: - From 41% at 4 weeks to 86% at 12 months
10	- Change in mean reported VAS (range 0-10) * OFC from 2.8 to 2.9 * DBPCFC from 4.5 to 4.1 - No significant difference	

Abbreviations: *A = Article number, AIT = Allergen Immunotherapy, BPFA = Birch pollen food allergy, DB-PCFC = Double-blind, placebo-controlled food challenge, ED = Eliciting dose , HD = Ingestion of highest dose without symptoms during the last performed challenge, N = Number, OFC = Oral food challenge, RCT = Randomized controlled trial, SCIT = Subcutaneous immunotherapy, SLIT = Sublingual immunotherapy, VAS = Visual analogue scale, vs = versus*

Table 4. Summary of the effect of birch pollen AIT on severity of symptoms during challenge and eliciting dose within treatment group (change from baseline) and between treatment and control group

Outcome measurement	**Severity of symptoms during challenge**		**Eliciting dose**	
Comparison of treatment group with:	Baseline	Control	Baseline	Control
Article number				
Studies with a moderate risk of bias				
1	++^	++^	++	
2				-
3	++	++	++	++
Studies with a high risk of bias				
4	+			-
5		NM		+
6*	++	++		++
7	++^	++^	++	++
8			++	
9**	++		++	
10				

Direction of effect: ● positive effect, ● no effect, ● negative effect

Size of effect in case a positive or negative effect was observed: < 20% difference + / - ; > 20% difference ++ / --; when the size of effect was not mentioned, the abbreviation NM was used

Abbreviations: *NM = not mentioned*

** Treatment group used OFC; control group used questionnaire*

*** At baseline questionnaire and at endpoint OFC*

^ *Only the objective symptoms are shown*

Supporting studies showed mostly positive effects

The seven studies with a high risk of bias were considered supportive, of which four[14–17] included a control group and three[18–20] did not (**Table 4**). When there was a positive effect on severity of symptoms during challenge, there was also a positive effect seen on the ED. Of the studies with a control group, three[15–17] showed a positive effect on severity of symptoms and the ED with mostly a large effect size and one[14] no effect on severity of symptoms during challenge and a negative effect with a small effect size on the ED. Of the studies without a control group, two[18,19] showed a positive effect with a large effect size and one[20] no effect.

Overall, these high risk of bias studies supported the moderately positive effect of birch pollen AIT on severity of symptoms during challenge and indicated that the effect on eliciting dose was more likely to be positive.

DISCUSSION

Due to the small number of included studies and the moderate to high risk of bias in these studies, this systematic review primarily shows that there is not enough evidence to conclude that AIT improves BPFA. There may be a positive effect on the severity of symptoms during challenge. The effect on eliciting dose is, however, unclear, and there is not enough information to draw a conclusion about the effect of birch pollen AIT on FA-QoL.

The evidence of the effect of birch pollen AIT on BPFA is of low quality

Seven[14–20] of the 10 included studies had a high risk of bias, mostly due to domain 2, 'Deviations from the intended interventions', followed by domain 4, 'Measurement of the outcome'. The high risk of bias in domain 2 was due to studies without a control group and studies with a control group but not adjusted for prognostic factors when no randomization was performed. The high risk of bias in domain 4 was attributed to the fact that, among others, studies performed an OFC only at the end of the study and not at the start[15,19] or used a method in the treatment group that was different from that in the control group[16]. Because most studies had a high risk of bias, the level of evidence was low. To obtain the best possible estimation of effectiveness, we decided to use the three studies with the lowest risk of bias for the assessment and the studies with a high risk of bias only as supporting evidence.

More research is needed to investigate the effectiveness of birch pollen AIT on BPFA

Birch pollen AIT seems to have a positive effect on BPFA as evidenced by an alleviation of symptoms during challenge, and it remains unclear if there is also a positive effect on the ED. That some studies found no or even a negative effect could be attributed to the fact that the included patients might not have had a pure BPFA, but in addition also a primary food allergy that did not improve or even worsen during the treatment and/or to the fact that there was an imbalance in the groups in this respect. Nowadays, by measuring the PR-10 as well as the non-PR-10 components, it is much better possible to differentiate between a pure BPFA and a primary food allergy.[21] The study that showed that there was no effect on symptoms during challenge and a negative effect on the eliciting dose measured only Cor a 1 and Cor a 8 but not Cor a 9 and 14.[12] Therefore, it was unsure whether only patients with pure PBFA were included.

Another reason could be that the follow-up period was too short. In general, the effect of immunotherapy increases with longer duration.[22,23] All three studies with the lowest risk of bias reported their results only after one year of AIT use.

Studies investigating the effect of birch pollen AIT on food allergy-related quality of life are lacking

Patient-reported outcome measures (PROMs) are often the best way of measuring patient symptoms and quality of life and can help reduce observer bias.[24] Unfortunately, only one study investigated the effect of birch pollen AIT on the patient reported outcome 'FA-QoL' using the validated FAQLQ-AF questionnaire and showed that there was no significant difference between patients receiving SCIT or placebo at endpoint.[11] As the primary burden on patients living with food allergy is reduced QoL, treatment success in trials should also be defined by improved QoL.[25,26] Future studies, investigating the effect of birch pollen AIT on BPFA, should therefore include QoL as an outcome.

It is not possible to evaluate potential differences between birch pollen SCIT and SLIT on BPFA

In total, six studies investigated the effect of birch pollen SCIT[11–13,16–18], three that of birch pollen SLIT[15,19,20] and one that of both birch pollen SCIT and SLIT[14] on BPFA. Because of the small number of studies and high risk of bias, there is too little evidence to draw a conclusion.

Effect of birch pollen on AIT on different foods

In our systematic review, we found that almost all studies investigated the effect of AIT with either hazelnut or apple as type of food. This is not surprising, as hazelnut and apple allergies are among the top three reported birch pollen-related food allergies in birch pollen endemic areas.[27] However, patients with BPFA are mostly allergic to multiple types of fruits, vegetables, and nuts. The effect on other foods still remains unknown. Therefore, it is important to evaluate the effect of AIT on a broader range of BPFA foods.

We expected a better therapeutic effect of birch pollen AIT on foods with PR-10 components that are more homologues to Bet v 1. However, although the PR-10 components of apple and hazelnut are more homologous to Bet v 1 than that of soy[28], this review showed a better effect of birch pollen AIT on soy[11] than on hazelnut allergy[12]. To confirm this hypothesis, more studies are needed that compare the effect of birch pollen AIT on multiple foods related to BPFA.

Further studies are needed to investigate the long-term effects of birch pollen AIT on BPFA

It is unknown how long the effects last after discontinuing AIT. Most of the included studies measured the outcome after 12 months of the start of birch pollen AIT, but none showed results after discontinuing of AIT. In 2003, Asero conducted a prospective cohort study to evaluate the long-term effect of birch pollen-specific SCIT on apple allergy after treatment cessation.[29] In this study, 21 BPFA patients who discontinued birch pollen SCIT could tolerate apple. However, the effect appeared to decrease over time, since after 30

months of discontinuation, only 52% of the patients remained symptom-free. Further studies are needed to investigate the long-term effects of birch pollen AIT on BPFA so that clinicians can advise patients appropriately.

Positive effect of other immunotherapies on plant food allergy

Besides the effect of birch pollen AIT on BPFA, several studies showed the effect of other immunotherapies on plant food allergy. Two studies reported about an effective treatment of birch pollen-related apple allergy. Kinacyian et al.[30] showed that patients receiving SLIT with rMal d 1 required a significantly higher dose of rMal d 1 to induce OAS compared to the group that received rBet v 1 and placebo ($p = 0.001$) and Kopac et al.[31] showed that apple consumption induces a transient tolerance. Furthermore, studies from Japan[32] and Italy[33] reported positive effects of Japanese cedar pollen-based SCIT and grass pollen SLIT, respectively, on plant food allergy. All of the above studies showed promising approaches for the effective treatment of plant food allergy, but these results should be confirmed before they are used in clinical practice.

Strengths and limitations

Due to the encountered high risk of bias and heterogeneity of the included studies in terms of, among other elements, the study design, type of food, type and dose of immunotherapy, and method of assessing response to treatment, it was not possible to pool the results. Furthermore, symptoms were often not specified, and none of the studies reported the minimal clinically important difference (MCID) to define improvement, which made it difficult to interpret whether the found differences were clinically relevant.[34] To provide the most reliable results, we focused on the three studies with the lowest risk of bias. Because of this selection, the number of patients was also small, with a total number of patients receiving AIT being 61 and those not receiving AIT being 37. However, the strengths of this review included its comprehensive search and methodological rigor, which also took into account patient-reported outcomes. This factor made this review the first systematic one to show the effect of BPFA on clinical and patient-reported outcomes.

Conclusions

To our knowledge, this is the first systematic review that evaluates the effect of birch pollen AIT on BPFA. Due to the low number of studies that fulfilled the inclusion criteria, a moderate to high risk of bias in these studies, and the low number of included patients per study, the level of evidence is low. The three studies with the lowest risk of bias showed that there might be a positive effect on severity of symptoms during challenge, but there was an unclear effect on eliciting dose and there was not enough information available to draw a conclusion about the effect of birch pollen AIT on FA-QoL. Taken together, no firm conclusion can be drawn and future research is warranted that uses robust clinical studies that take into account abovementioned aspects, including the long-term effects.

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SUPPLEMENTAL

Supplemental 1.A Search strategy PubMed

Search strategy for PubMed search performed on November 3rd 2022.

Patient population: Birch pollen-related food allergy

1. food*[Title/Abstract] OR fruit[MeSH Terms] OR fruit*[Title/Abstract] OR rosaceae[MeSH Terms] OR rosaceae*[Title/Abstract] OR apple*[Title/Abstract] OR malus*[Title/Abstract] OR apricot*[Title/Abstract] OR cherr*[Title/Abstract] OR peach*[Title/Abstract] OR plum*[Title/Abstract] OR nectarin*[Title/Abstract] OR prunus*[Title/Abstract] OR pear*[Title/Abstract] OR pyrus*[Title/Abstract] OR actinidia[MeSH Terms] OR actinidia*[Title/Abstract] OR kiwi*[Title/Abstract] OR mangifera[MeSH Terms] OR mangifera*[Title/Abstract] OR mango*[Title/Abstract] OR diospyros[MeSH Terms] OR diospyros*[Title/Abstract] OR artocarpus[MeSH Terms] OR artocarpus*[Title/Abstract] OR jackfruit*[Title/Abstract] OR litchi[MeSH Terms] OR lychee*[Title/Abstract] OR litch*[Title/Abstract] OR leechee*[Title/Abstract] OR vitis[MeSH Terms] OR vitis*[Title/Abstract] OR grape*[Title/Abstract] OR ficus[MeSH Terms] OR ficus*[Title/Abstract] OR fig*[Title/Abstract] OR fabaceae[MeSH Terms] OR fabaceae*[Title/Abstract] OR legume*[Title/Abstract] OR soy food[MeSH Terms] OR soybeans[MeSH Terms] OR soy*[Title/Abstract] OR soj*[Title/Abstract] OR bean*[Title/Abstract] OR vegetable[MeSH Terms] OR vegetable*[Title/Abstract] OR daucus carota[MeSH Terms] OR daucus carota*[Title/Abstract] OR carrot*[Title/Abstract] OR apium graveolens[MeSH Terms] OR apium graveolen*[Title/Abstract] OR celer*[Title/Abstract] OR nuts[MeSH Terms] OR nut[Title/Abstract] OR nuts[Title/Abstract] OR corylus[MeSH Terms] OR corylus*[Title/Abstract] OR hazelnut*[Title/Abstract] OR arachis[MeSH Terms] OR arachis*[Title/Abstract] OR peanut*[Title/Abstract] OR solanum tuberosum[MeSH Terms] OR solanum tuberosum*[Title/Abstract] OR spices[MeSH Terms] OR spice*[Title/Abstract] OR herb*[Title/Abstract] OR sunflower seed*[Title/Abstract]
2. hypersensitivities[MeSH Terms] OR hypersensitiv*[Title/Abstract] OR allergens[MeSH Terms] OR allerg*[Title/Abstract] OR cross reactions[MeSH Terms] OR cross react*[Title/Abstract] OR crossreact*[Title/Abstract] OR ige mediat*[Title/Abstract] OR sensitis*[Title/Abstract] OR sensitis*[Title/Abstract]
3. 1 AND 2
4. food hypersensitivities[MeSH Terms]
5. 3 OR 4

6. pollen[MeSH Terms] OR pollen*[Title/Abstract] OR trees[MeSH Terms] OR tree*[Title/Abstract] OR orchard*[Title/Abstract] OR plane*[Title/Abstract] OR betulaceae[MeSH Terms] OR alnus*[Title/Abstract] OR alder*[Title/Abstract] OR betula*[Title/Abstract] OR birch*[Title/Abstract] OR corylus*[Title/Abstract] OR hazel*[Title/Abstract] OR

filbert*[Title/Abstract] OR hornbeam*[Title/Abstract] OR quercus[MeSH Terms] OR quercus*[Title/Abstract] OR oak*[Title/Abstract]
7. 5 AND 6

8. oral allergy syndrom*[Title/Abstract] OR pollen food syndrom*[Title/Abstract] OR pollen-food syndrome*[Title/Abstract] OR pollen-related food*[Title/Abstract] OR pollen related food*[Title/Abstract]
9. 7 OR 8

Intervention: Subcutaneous and/or sublingual immunotherapy

10. immunotherapy[MeSH Terms] OR immunotherap*[Title/Abstract] OR oral[Title/Abstract] OR injection*[MeSH Terms] OR subcutaneous*[Title/Abstract] OR sublingual immunotherapy[MeSH Terms] OR administration sublingual[MeSH Terms] OR sublingual*[Title/Abstract] OR SCIT[Title/Abstract] OR SLIT[Title/Abstract]
11. 6 AND 10
12. 9 AND 11

Supplemental 1.B Search strategy Embase

Search strategy for Embase search performed on November 3rd 2022.

Patient population: Birch pollen-related food allergy

1. food*:ab,ti OR 'fruit' OR fruit*:ab,ti OR 'rosaceae' OR rosaceae*:ab,ti OR 'apple' OR 'malus' OR 'apricot' OR 'cherry' OR 'peach' OR 'plum' OR 'nectarine' OR 'prunus' OR 'pear' OR 'pyrus' OR 'actinidia' OR actinidia:ab,ti OR 'kiwifruit' OR kiwi:ab,ti OR 'mangifera' OR mangifera:ab,ti OR 'mango' OR 'diospyros' OR diospyros*:ab,ti OR 'artocarpus' OR artocarpus:ab,ti OR jackfruit:ab,ti OR 'lychee' OR litchi:ti,ab OR litch*:ti,ab OR leechee*:ab,ti OR 'vitis' OR vitis*:ab,ti OR 'grape' OR grape:ab,ti OR 'ficus' OR ficus*:ab,ti OR fig*:ab,ti OR 'fabaceae' OR fabaceae:ab,ti OR 'legume' OR legume*:ab,ti OR 'soy food' OR 'soybean' OR soy*:ti,ab OR soj*:ab,ti OR 'bean' OR 'vegetable' OR vegetable:ab,ti OR 'carrot' OR carrot*:ab,ti OR 'celery' OR celery:ab,ti OR 'nut' OR nut*:ab,ti OR 'hazelnut' OR corylus*:ab,ti OR 'arachis' OR arachis*:ab,ti OR 'peanut' OR peanut:ab,ti OR 'potato' OR tuberosum*:ab,ti OR 'spice' OR spice*:ab,ti OR 'herb' OR sunflowerseed*:ab,ti
2. 'hypersensitivity' OR hypersensitiv*:ab,ti OR 'allergen' OR allerg*:ab,ti OR 'cross reaction' OR crossreact*:ab,ti OR 'immunoglobulin E' OR 'immediate type hypersensitivity' OR 'sensitization' OR sensitis*:ti,ab
3. 1 AND 2
4. 'food allergy'
5. 3 OR 4

6. 'pollen' OR pollen:ab,ti OR 'tree' OR tree*:ab,ti OR 'orchard' OR plane:ab,ti OR 'betulaceae' OR alnus*:ab,ti OR 'alder' OR betula*:ab,ti OR 'birch' OR 'hazelnut' OR corylus*:ab,ti OR hazel*:ab,ti OR filbert*:ab,ti OR hornbeam*:ab,ti OR 'oak' OR quercus*:ab,ti
7. 5 AND 6
8. 'oral allergy syndrome' OR 'pollen food syndrome' OR 'pollen-food syndrome*':ab,ti OR 'pollen-related food*':ab,ti OR 'pollen related food*':ab,ti
9. 7 OR 8

Intervention: Subcutaneous and/or sublingual immunotherapy

10. 'immunotherapy' OR immunotherap*:ab,ti OR oral*:ab,ti OR 'injection' OR subcutaneous*:ab,ti OR 'subcutaneous drug administration' OR 'sublingual immunotherapy' OR 'sublingual drug administration' OR sublingual*:ab,ti OR 'subcutaneous immunotherapy' OR scit*:ab,ti OR slit*:ab,ti
11. 6 AND 10
12. 9 AND 11

Supplemental 1.C Search strategy Cochrane

Search strategy for Cochrane search performed on November 3rd 2022.

Patient population: Birch pollen-related food allergy

1. [mh 'soy foods'] or [mh dauces carota] or [solanum tuberosum] or [mh fruit] or [mh artocarpus] or [mh rosacea] or [mh litchi] or [mh actinidia] or [mh vitis] or [mh mangifera] or [mh ficus] or [mh malus] or [mh fabaceae] or [mh diospyros] or [mh soybeans] or [mh vegetable] or [mh apium] or [mh nuts] or [mh corylus] or [mh arachis] or [mh spices] or (food* or fruit* or rosaceae* or apple* or malus* or apricot* or cherr* or peach* or plum* or nectarin* or prunus* or pear* or pyrus* or actinidia* or kiwi* or mangifera* or mango* or diospyros* or artocarpus* or jackfruit* or lychee* or litch* or leechee* or vitis* or grape* or ficus* or fig* or fabaceae* or legume* or soy* or soj* or bean* or vegetable* or ''daucus carota''* or carrot* or ''apium graveolen''* or celer* or nut or nuts or corylus* or hazelnut* or arachis* or peanut* or ''solanum tuberosum''* or spice* or herb* or ''sunflower seed''*):ti,ab,kw
2. [mh 'cross react'] or [mh hypersensitivity] or [mh allergens] or (hypersensitiv* or ''cross react*'' or ''ige mediat*'' or allerg* or crossreact* or sensitis*):ti,ab,kw
3. 1 AND 2
4. [mh food hypersensitivity]
5. 3 OR 4
6. [mh pollen] or [mh trees] or [mh betulaceae] or [mh quercus] or (pollen* or tree* or orchard* or plane* or alnus* or alder* or betula* or birch* or corylus* or hazel* or filbert* or hornbeam* or quercus* or oak*):ti,ab,kw

7. 5 AND 6
8. ("oral allergy syndrome" or ''pollen food syndrome" or ''pollen-food syndrome"* or ''pollen-related food"* or ''pollen related food"):ti,ab,kw
9. 7 OR 8

Intervention: Subcutaneous and/or sublingual immunotherapy

10. 'immunotherapy' OR immunotherap*:ti,ab,kw OR oral*:ti,ab,kw OR 'injections' OR subcutaneous*:ti,ab,kw OR 'injections, subcutaneous' OR 'sublingual immunotherapy' OR 'administration, sublingual' OR sublingual*:ti,ab,kw OR scit*:ti,ab,kw OR slit*:ti,ab,kw
11. 6 AND 10
12. 9 AND 11

11

Birch pollen SLIT is effective in birch pollen-related food allergy and improves quality of life

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Under review

Introduction

Birch pollen-related food allergy (BPFA) is the most common food allergy in Northern- and Western Europe, yet no treatment is available. In BPFA patients, we investigated the effectiveness of birch pollen sublingual immunotherapy (SLIT) on health-related quality of life (HRQL) and (predictors for) clinical response.

Methods

Patients aged 18-65 years with birch pollen allergy and confirmed BPFA by food challenge and sensitization, were included. After one year of birch pollen SLIT, treatment effects were assessed. Primary endpoint was an improvement of ≥ 0.5 points in total Food Allergy Quality of Life Questionnaire-Adult Form (FAQLQ-AF) score. Secondary endpoints included clinical response defined as either an increase in threshold during FC or improvement in severity at the same threshold, for at least one food. Other secondary endpoints included changes in sIgE levels. Predictors associated with response were analyzed using logistic regression.

Results

Of 48 patients screened, 42 were included and 37 completed one-year follow-up. After one year, 50.0% (95%CI: 31.0%-76.4%) showed clinically relevant improvement in FAQLQ-AF. Furthermore, 66.6% were clinical responders. Among responders, no deterioration in threshold or severity was observed for other foods, and 39.3% became tolerant. sIgE levels increased at three months but returned to baseline after one year. No variables were significantly associated with an improvement in FAQLQ-AF. Baseline severity during challenge predicted clinical response (OR=6.78; 95%CI 1.03-44.70).

Conclusion

One year of birch pollen SLIT improved HRQL in half of BPFA patients and induced a positive clinical response in two-thirds. Higher baseline severity predicted clinical response.

INTRODUCTION

Birch pollen allergy is common in Northern and Central Europe.[1] The major allergen, Bet v 1, cross-reacts with PR-10 proteins in plant foods, leading to birch pollen related food allergy (BPFA). BPFA symptoms are typically mild. However, more severe reactions, including anaphylaxis, are reported.[2–4]

Beyond clinical symptoms, food allergy impacts health related quality of life (HRQL) through dietary and social restrictions, as well as emotional and social burden.[5] Patients with BPFA are often allergic to a wide range of plant-based food (range between 4-13[6]), forcing them to avoid many nutritious foods. Furthermore, the potential for moderate to severe allergic reactions, though less frequent, may contribute to an increased disease burden. HRQL In patients with BPFA is on average moderately impaired.[6,7] Since improving HRQL is important for individuals with FA, clinical trials should also measure HRQL.[8,9]

Despite its high prevalence and broad clinical manifestation, there is currently no curative treatment available for BPFA.[10] Since birch pollen is the primary sensitizer in BPFA patients, birch pollen allergen immunotherapy (AIT) may also benefit patients with BPFA.[11] However, a recent review concluded that the current evidence is insufficient to draw conclusions regarding the effectiveness of birch pollen AIT for BPFA.[10]

Therefore, a three year longitudinal daily practice study was started to evaluate the effect of birch pollen SLIT on HRQL and clinical response during challenges in patients with birch pollen allergy and concomitant BPFA. We also determined predictors at baseline for a response in HRQL and clinical response.

METHODS

Study population

Patients aged 18–65 years visiting the Department of Dermatology and Allergology at the University Medical Center Utrecht (UMCU), The Netherlands, were recruited between September 2022-December 2024. Eligible patients had a birch pollen-induced allergic rhinitis (AR) with an indication for birch pollen SLIT, and concomitant birch pollen-related food allergy (BPFA).

BPFA was defined by:

1. Symptoms and sensitization to birch pollen extract and/or Bet v 1 (skin prick test [SPT] [ALK, Madrid, Spain] or specific IgE [sIgE]), and
2. Positive challenge for at least one of the following foods: almond, apple, carrot, cherry, hazelnut, kiwi, peach, plum, walnut, or soymilk, accompanied by sensitization to that food's extract (via SPT, prick to prick test [PTP], or ImmunoCAP [ThermoFisher Scientific, Uppsala, Sweden]). Sensitization to the associated PR-10 component (e.g., Cor a 1 for hazelnut), without sensitization to the associated non-PR-10 components (e.g., Cor a 9) were required when available, and
3. Customized immunoblots (EUROLINE, EUROIMMUN, Lübeck, Germany) confirming sensitization to PR-10 allergens (Mix with Bet v 1, Que a 1, Mal d 1, Jug r 5, Ara h 8), and absence of sensitization to lipid transfer protein (LTP) allergens (Mix with Pru p 3, Tri a 14, Pha v 3, Lac s 1, Mal d 3, Jug r 3, Art v 3, Cor a 8, Ara h 9), nut seed storage protein (SSP) allergens (Mix with Ana o 3, Jug r 1, Ber e 1) and peanut SSP allergens (Mix with Ara h 1, 2, 3, 6, 7).

Exclusion criteria contained contraindications for birch pollen SLIT, prior birch pollen allergen immunotherapy (AIT) within three years, insufficient Dutch language proficiency, and/or chronic spontaneous urticaria and/or angioedema.

The study was approved by the local Medical Research Ethics Committee (protocol number 22-819). All patients provided written informed consent.

Treatment

Patients received ITULAZAX birch pollen SLIT-tablets (ALK-Abelló, Hørsholm, Denmark), indicated for moderate-to-severe allergic rhinitis and/or conjunctivitis caused by birch-homologous tree pollen in patients insufficiently controlled by symptomatic medication.[12,13] It is a sublingual lyophilizate containing 12 SQ-Bet units, self-administered once daily. The first dose was administered under medical supervision at the hospital.[14] Patients who initially experienced difficulty tolerating the full dose followed a step-up dosing schedule.

Data collection

Health-related quality of life

HRQL was assessed using the Food Allergy Quality of Life Questionnaire-Adult Form (FAQLQ-AF), comprising 29 questions across four domains: 1) allergen avoidance and dietary restrictions, 2) emotional impact, 3) risk of accidental exposure and 4) food allergy related health.[15] Scores range from 1-7; higher scores indicate greater impairment.

The minimal important difference (MID) is a reduction of 0.5 points in the total FAQLQ-AF score.[16] Patients completed the FAQLQ-AF at baseline and one year.

Sensitization tests

Sensitization to birch pollen and BPFA-related foods was assessed using SPT, PTP, and sIgE tests. For all patients, SPT was performed using extracts for almond, apple, bell pepper, carrot, celery, cherry, hazelnut, navy bean, pecan, peanut, soy, string bean, and walnut. PTP were performed for apple, kiwi, peach, and tomato. Specific IgE was measured to Bet v 1, apple, Mal d 1, hazelnut, Cor a 1, kiwi, Act d 8, peach, Pru p 1, peanut, Ara h 8, celery, Api g 1, soy, Gly m 4, and walnut. For soy-related symptoms, PTP was also done with soy milk, tofu, and tempeh. All tests were performed at baseline and after one year, and sIgE levels were also measured after three months. A wheal diameter ≥ 3 mm and sIgE levels ≥ 0.35 kU_A/L were considered positive, respectively.

Furthermore, to accurately screen and select eligible patients at baseline, sIgE to ImmunoCAP Cor a 9, Cor a 14, Pru p 3, Ara h 2, Ara h 6, Gly m 5, and Gly m 6 and immunoblots with PR-10-, LTP-, nut SSP-, and peanut SSP- mixes were measured for all patients (see BPFA definition). Immunoblot analyses followed manufacturer instructions, using EUROBlotOne system and EUROLineScan software. Results of ≥ EAST class 1 were considered positive.

Oral food challenge

Patients performed an oral food challenge in the hospital (OFC) or at home (HOFC) (henceforward together referred as challenges) for at least one BPFA-related food. OFC were conducted in case of prior severe symptoms or patient preference, otherwise, HOFC was performed when deemed safe. Protocols followed PRACTALL guidelines and expert opinion,[17] adapted for home use with clear instructions. For nuts, the protocol included the following steps: 3 mg, 10 mg, 30 mg, 100 mg, 300 mg, 1000 mg and 3000 mg protein. For fruit and vegetables, the protocol included the following steps: holding ½ teaspoon in the mouth for one minute without chewing or swallowing, followed by consuming ½ teaspoon, 1 teaspoon, 1/8 portion, 1/4 portion, 1/2 portion, and finally 1 whole portion of the challenged food. For soymilk, the following steps were conducted: 0.1 ml, 0.3 ml, 1 ml, 2.2 ml, 6.5 ml, and 21.7 ml soymilk administered in a mixture, followed by open-label doses of 15 ml, 45 ml, and 135 ml of soymilk. Every 20-30 minutes a new dose was administered. Raw and/or processed foods were selected based on symptom history.

Challenges were positive if objective allergic reactions occurred or persistent subjective symptoms lasted > 45 minutes. An expert panel assessed outcomes and severity.

Subjective and objective threshold levels were determined.

Severity

The overall severity of a food allergic reaction during challenge was classified according to the oFASS-5 severity score.[18] This score was categorized into five different grades: oFASS-5 grade 1 includes oropharyngeal symptoms; grade 2 includes symptoms of one of the following organ system: mucosa, eyes, nose, skin, or gastrointestinal tract with or without grade 1 symptoms; grade 3 includes symptoms of at least two of grade 2 organ systems with or without grade 1 symptoms; grade 4 includes respiratory symptoms with or without grade 1, 2 or 3 symptoms, and grade 5 includes cardiovascular symptoms with or without grade 1, 2, 3 or 4 symptoms. The grades were classified as mild for oFASS-5 grade 1, moderate for oFASS-5 grades 2 or 3, and severe for grades 4 or 5.

Outcome measures

Our primary endpoint was patient-reported response, defined as an improvement of ≥ 0.5 points in the total FAQLQ-AF score. The main secondary endpoint included clinical response, defined as either 1) an increase in threshold during challenge or 2) an improvement in severity during challenge at the same threshold, for at least one food. Additional secondary outcomes included changes in sIgE levels, SPT wheal sizes, and changes in threshold and severity per food

Statistical analysis

Sample size

To ensure that the observed treatment effect was not primarily due to placebo effects or regression to the mean, the primary response rate was compared to a maximum expected placebo response rate for clinically relevant improvement in HRQL. Since only one study has investigated the effect of birch pollen AIT on FAQLQ-AF in patients with BPFA reporting no change in the placebo group[7], and no other studies in adults were available, data in children were used as a reference. Only one study reported a placebo response rate for HRQL improvement, which was 5%.[19] Other studies only reported non-significant changes in HRQL, with mean changes in FAQLQ-AF total score from baseline between 0.01 - 0.40 in the placebo groups.[20–22] Based on these data and expert opinion, a maximum 20% placebo response was assumed. We assumed the treatment group to show at least a 20% higher response rate (i.e. 40%). To test whether the response rate exceeds 20%, with $\alpha = 0.05$ and 80% power, 36 patients are needed. To account for potential missing data or dropouts, 45 patients were included.

Statistics

Patients' characteristics were presented as means with standard deviations (SD) for normally distributed data, and as medians with interquartile ranges (Q1, Q3) for not normally distributed data. Frequency and percentage were used to describe categorical variables.

The percentage of patients with a response in HRQL or clinical response was calculated with corresponding 95% confidence interval (CI). Those without 12-month data, were classified as non-responder. For the primary endpoint, the effect of SLIT was considered statistically significant if the lower bound of the CI exceeded the predefined 20% efficacy limit.

Changes in continuous and ordinal variables between baseline and one year were described using mean with SD, or median with interquartile range and tested using the paired t-tests or Wilcoxon signed rank tests. To account for multiple challenges per patient, change scores were first averaged per patient. For dichotomous variables (e.g. positive challenge), first, the number of positive outcomes was divided by the number of challenges in a patient (i.e. proportion positive outcomes per patient) and tested with the Wilcoxon signed rank test.

Univariable logistic regression was used to assess associations between a successful response in HRQL or a clinical response and factors including demographics, allergic co-morbidities, baseline sIgE levels, baseline SPT/PTP results, and baseline challenge results. Analyses were conducted for all patients for whom information on response was available. Variables with p-value < 0.20 in univariable analyses or deemed clinically relevant up to a maximum of 1 predictor per five responders in the study population were then combined in multivariable analysis.

Analyses were performed using IBM SPSS Statistics version 30.0.0.0. P-values ≤ 0.05 were considered statistically significant unless stated otherwise.

RESULTS

Patient characteristics

Of 48 screened patients, 42 were included. During the first year, five patients discontinued SLIT: three due to side effects, and two due to unrelated health issues. In total, 37 patients completed the one-year follow-up period. The mean age was 35 and 62% were female (**Table 1**). The median FAQLQ-AF total score at baseline was 3.4, indicating a moderate to severe impact on HRQL.

At baseline, 91 challenges were positive, most commonly with apple (N = 26) and one of the hazelnut products (N=43) (**Figure 1**).

Table 1. Baseline characteristics

Baseline characteristics		
Age (mean, SD)	34.81	(12.15)
Sex; female (N, %)	26	(61.9)
Allergic comorbidities		
Allergic rhinitis (N, %)	42	(100)
Allergic asthma (N, %)	17	(40.5)
Atopic dermatitis (N, %)	21	(50.0)
FAQLQ-AF (median, Q1-Q3)		
Total score	3.4	(2.2-4.4)
Allergen avoidance and dietary restrictions	3.5	(2.2-4.7)
Emotional impact	3.4	(2.2-4.7)
Risk of accidental exposure	2.8	(1.8-4.1)
Food allergy-related health	3.7	(2.5-4.7)

Abbreviations: FAQLQ-AF = food allergy quality of life questionnaire – adult form

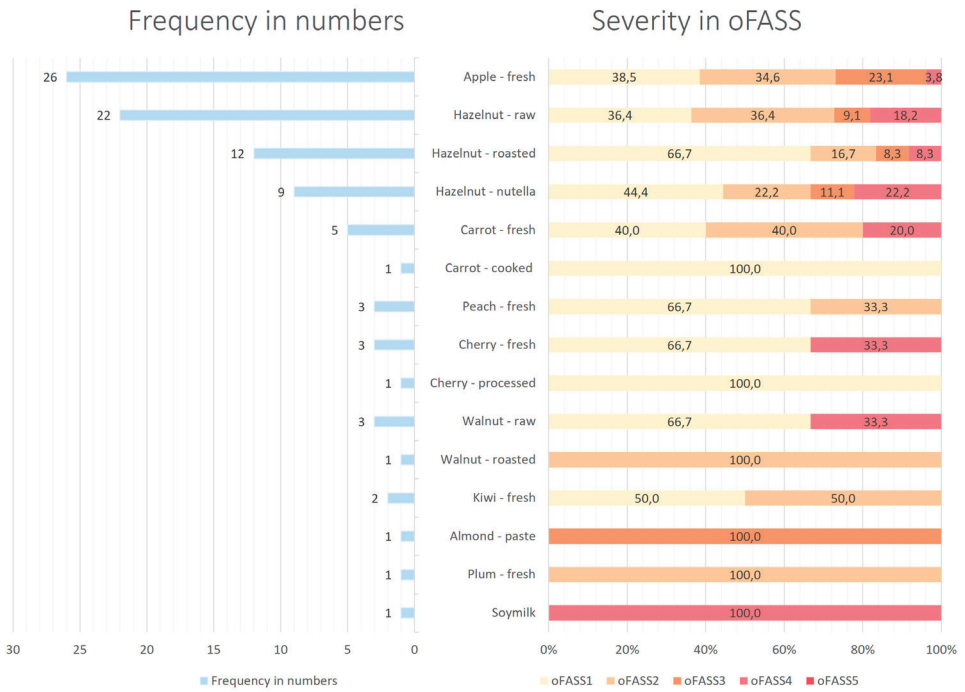


Figure 1. Frequency and severity of food challenges at baseline

Half of the patients show improvement in health-related quality of life

After one year, the primary endpoint, i.e. an improvement in FAQLQ-AF total score of ≥ 0.5, was reached by 50.0% of patients (95% CI 31.0%-76.4%) (**Figure 2**). Domain-specific response rates were 61.9% (95% CI: 40.4% – 90.7%) for food allergy-related health, 52.4% (95% CI: 32.8% – 79.3%) for emotional impact, 45.2% (95% CI: 32.8% – 79.3%) for allergen avoidance and dietary restrictions, and 40.5% (95% CI: 23.6% – 64.8%) for risk of accidental exposure. All response rates exceeded the estimated maximum placebo response of 20% and demonstrate meaningful HRQL improvements across all FAQLQ-AF domains.

Significant improvements were also observed in the total FAQLQ-AF score and all four domains (**Figure 3**). The median total score decreased from 3.4 to 2.5, corresponding to an average improvement of 0.6 points ($p < 0.001$). The greatest improvement was observed in the domain food allergy-related health, which declined by 0.9 points ($p < 0.001$), while the smallest improvement occurred in the domain risk of accidental exposure (0.4 points, $p = 0.006$).

Clinical response observed in two thirds of patients

A positive clinical response was observed for 66.7% (95%CI: 44.3%-96.4%) of patients. Among these responders, 39.3% of patients became tolerant and none showed a deterioration in one of the other foods. Among patients without a clinical response, deterioration for at least one food

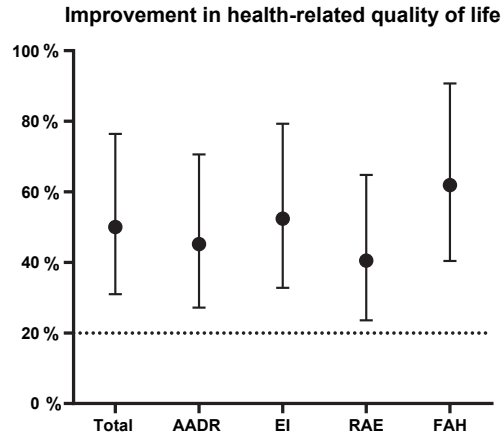


Figure 2. Percentage of patients with an improvement of ≥ 0.5 points in FAQLQ-AF total and score domain scores, with 95% confidence intervals
Abbreviations: AADR = Allergen Avoidance and Dietary Restrictions; EI = Emotional Impact; RAE = Risk of Accidental Exposure; FAH = Food Allergy–Related Health
Dotted line presents the estimated maximum placebo response

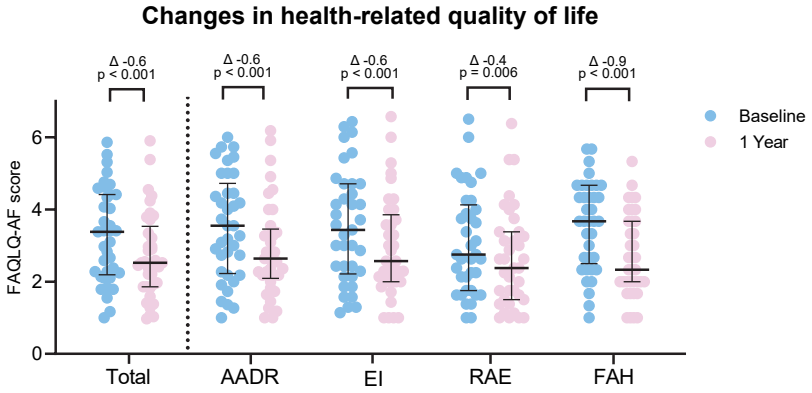


Figure 3. FAQLQ-AF total and domain scores at baseline and one year, lines and error bars represent the medians with interquartile ranges

Abbreviations: AADR = Allergen Avoidance and Dietary Restrictions; EI = Emotional Impact; RAE = Risk of Accidental Exposure; FAH = Food Allergy–Related Health

was observed in 21.4% (95% CI: 9.8%-40.7%). However, since these results included the five patients who discontinued the study and were counted as non-responders, when considering only the 37 patients who completed the one year follow-up, a clinical response was observed in 75.7% and a deterioration only in 10.8%.

The challenge results of the individual foods are presented in **Figure S1-S3**, which illustrated the changes in subjective and objective threshold, as well as severity, for the individual foods.

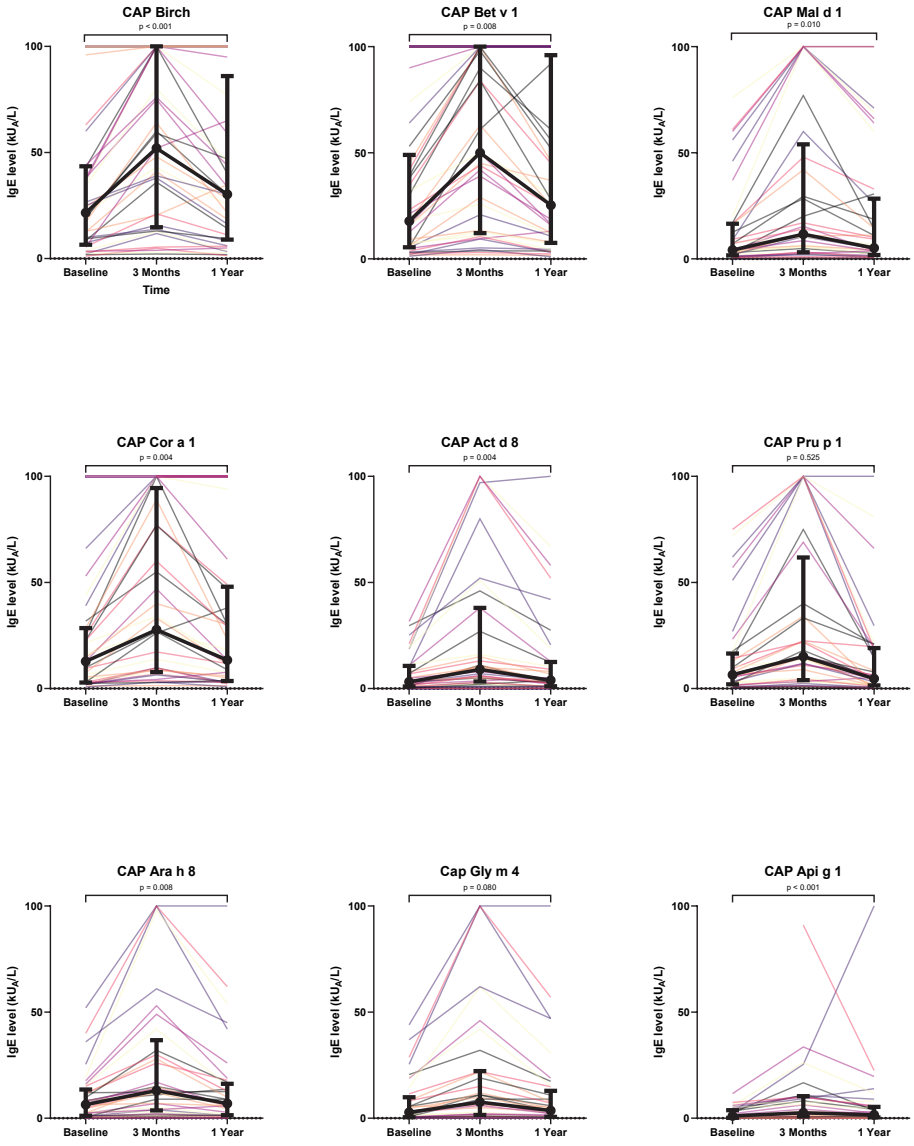


Figure 4. Specific IgE to birch and PR-10 components with mean and interquartile ranges at baseline, three months and one year

IgE levels increased after three months and returned to baseline values after one year

Specific IgE levels at baseline, 3 months, and 1 year are shown in **Figure 4**. At three months, sIgE levels to all tested PR-10 components doubled or even tripled compared to baseline. By one year, these levels declined and returned to values close to baseline.

SPT and PTP wheal sizes at baseline are shown in **Table S1**. After one year of treatment, a general trend toward reduced wheal sizes was observed, with only statistically significant decreases for birch, almond, carrot, and walnut.

These changes in immunologic markers provide supporting evidence for the clinical efficacy of birch pollen SLIT.

Predictors of a successful response to birch pollen SLIT

In the univariate analyses, no associations were found between any of the variables evaluated and an improvement in HRQL (**Table 2**). Only a higher severity during the baseline challenge was significantly associated with a clinical response after one year of treatment (OR= 6.8; 95% CI 1.0-44.7).

In the multivariate analyses, the variables 'severity during baseline challenge' and 'baseline sIgE level to Bet v 1', were used in a multivariable logistic regression analysis. In this model, severity during baseline challenge was the only variable significantly associated with a clinical response with an OR of 6.78 (95% CI 1.03-44.70).

DISCUSSION

After one year of birch pollen SLIT, a clinically relevant improvement in HRQL was observed in 50.0% of patients and a positive clinical response based on challenges in 66.7% of patients. Changes in immunological markers provide supporting evidence for the clinical efficacy of birch pollen SLIT. Furthermore, we demonstrated that a higher severity at baseline challenge was associated with patients more likely to respond to birch pollen SLIT.

Improvement in health-related quality of life in half of the patients

After one-year of birch pollen SLIT, there was a significant clinical relevant improvement in HRQL in half of the patients. These results were based on a worst-case scenario where all five patients lost to follow-up were classified as non-responders. Restricting the analysis to the 37 patients who completed the one-year follow-up, this even increased to 56.8%.

To our knowledge, no other studies have investigated the effect of birch pollen allergen immunotherapy (AIT) on HRQL in patients with BPFA as comprehensively as this study. Only one study examining subcutaneous immunotherapy (SCIT) with Bet v 1 in patients with soy allergy and

Table 2. Univariate analysis of predictors for health related quality of life and clinical response

	Response in HRQL			Clinical response		
	OR	**95% CI**		**OR**	**95% CI**	
Demographics						
age; mean sd	1.1	1.0	1.1	1.0	0.9	1.1
sex; N%	0.8	0.2	3.0	2.4	0.4	12.9
AD; N%	0.9	0.3	3.2	3.3	0.6	20.2
AA; N%	0.5	0.1	1.9	0.9	0.2	4.6
Baseline IgE levels (median + IQR)						
birch	1.0	1.0	1.0	1.0	1.0	1.0
Bet v 1	1.0	1.0	1.0	1.0	1.0	1.5
apple	0.9	0.7	1.2	1.3	0.7	2.4
Mal d 1	1.0	1.0	1.0	1.0	1.0	1.1
Kiwi	1.3	0.7	2.3	1.0	0.7	1.6
Act d 8	1.0	0.9	1.1	1.0	0.9	1.1
Peach	1.0	0.8	1.1	1.1	0.9	1.4
Pru p 1	1.0	1.0	1.0	1.0	1.0	1.1
Peanut	1.1	0.8	1.6	2.7	0.4	16.3
Ara h 8	1.0	1.0	1.1	1.1	0.9	1.3
Soy	1.0	0.1	8.2	325.6	0.0	45384783.9
Gly m 4	1.0	1.0	1.1	1.3	0.9	1.9
Hazelnut	1.0	1.0	1.0	1.0	1.0	1.1
Cor a 1	1.0	1.0	1.0	1.0	1.0	1.0
Selery	0.7	0.5	1.1	1.4	0.7	2.9
Api g 1	0.9	0.7	1.2	1.0	0.8	1.4
Cherry	0.9	0.7	1.2	1.2	0.8	2.0
Baseline wheal sizes (median + IQR)						
Birch	0.9	0.6	1.2	0.9	614.0	1.4
Almond	1.1	0.8	1.4	0.9	0.7	1.2
Hazelnut	1.0	0.8	1.4	1.1	0.7	1.6
Peanut	0.9	0.7	1.2	1.2	0.8	1.7
Celery	0.8	0.5	1.3	0.9	0.5	1.4
Soy	1.1	0.7	1.6	0.9	0.5	1.4
Walnut	1.1	0.8	1.7	0.8	0.5	1.3
Carrot	0.9	0.7	1.2	1.1	0.8	1.5
Apple	1.0	0.9	1.2	1.1	0.8	1.3
Soy milk	0.6	0.3	1.2	1.0	0.4	2.1
Tempeh	0.5	0.2	1.6	0.9	0.2	3.8
Tofu	0.7	0.4	1.3	1.4	0.3	6.4
Kiwi	1.0	0.9	1.2	1.0	0.8	1.2
Peach	1.1	0.9	1.4	0.9	0.6	1.2

Table 2. Univariate analysis of predictors for health related quality of life and clinical response (*continued*)

	Response in HRQL			Clinical response		
	OR	**95% CI**		**OR**	**95% CI**	
Challenge baseline						
oFASS; median IQR	0.8	0.4	1.6	**6.8**	**1.0**	**44.7**
threshold sub; median IQR	1.1	0.7	1.7	1.3	0.7	2.6
threshold obj; median IQR	1.0	0.7	1.4	0.4	0.1	1.4

Values in bold indicate statistical significance. *Abbreviations: CI = confidence interval; OR = odds ratio*

noted that the FAQLQ-AF total score remained nearly unchanged in the treatment and placebo group.[7] Another study in adults investigating oral immunotherapy for milk, egg, peanut, sesame, and tree nuts and showed significant improvement in the treatment group with no change in FAQLQ-AF total score in the control group after a median duration of eight months.[23] Therefore, the improvement in HRQL observed in our study is likely beyond what can be attributed to a placebo effect.

These findings highlight the potential of birch pollen SLIT to meaningfully improve HRQL in patients with BPFA.

Two third of patients had a positive clinical response

In total, 66.7% of patients had a clinical response, i.e. an improvement in threshold or severity, of which 39.3% reached complete tolerance after already one year.

A recent review of ten studies demonstrated overall only a moderately positive effect on reaction severity and an unclear effect on eliciting dose.[10] The fact that included studies showed no effect, or even negative outcomes, may be due to inclusion of patients with primary food allergies in addition to BPFA. To address this, we applied strict inclusion criteria based on detailed sensitization profiles, enrolling only patients who were positive for PR-10 components and in addition negative for non-PR-10 components, to ensure as far as possible that the symptoms were really related to BPFA and not to primary allergens. This may explain the more favorable outcomes observed in our study, and may emphasize the importance of precise patient profiling and selection in future research on immunotherapy for BPFA.

It is possible that SLIT exerts more favorable effects due to its local immunomodulatory actions at the site of allergen administration, namely the oral mucosa, where the immune response is initially triggered. As far as known, only three studies investigated the effect of birch pollen SLIT on BPFA.[24–26] Of these, two showed a positive effect[24,25] and one no change.[26] However, both studies that demonstrated improvement did not perform OFCs at baseline, limiting the interpretation of their findings.

Predicting response

We showed that a higher severity during baseline challenge was the only variable significantly associated with a positive clinical response. To our knowledge, no studies described the relationship between baseline severity and efficacy of AIT in BPFA. However, a recent study on SLIT for house dust mite allergic rhinitis demonstrated that higher baseline symptom scores significantly predicted better clinical response.[27] Therefore, patients with severe symptoms in their clinical history may be more responsive to the effects of immunotherapy. Identifying such predictors could support more personalized treatment approaches and help optimize patient selection for SLIT.

Strengths and limitations

The main limitation of this study is the absence of a placebo group, as it was a daily practice study. This makes it more challenging to distinguish between natural variation or regression to the mean of symptoms over time and the true treatment effect of birch pollen SLIT. While HRQL is a subjective measure and potentially susceptible to placebo effects, the magnitude of improvement observed in our study exceeded the a priori defined maximum expected placebo response. As we included all consecutive patients with a doctors diagnosed BPFA, the placebo effect was probably limited in this study, and thus the results likely represent a true effect of birch pollen SLIT in our study. Moreover, the parallel improvements seen during challenges and the immunologic changes lend additional support to the clinical relevance of our findings. Secondly, the relatively small sample size limits statistical power for subgroup analyses. However, this study is the first to investigate multiple BPFA-related foods with challenges, providing a unique opportunity to comprehensively assess the clinical effects of birch pollen SLIT across a range of relevant allergens.

One of the main strengths of this study is the thorough selection of patients to create an as pure as possible BPFA group, minimizing confounding from primary food allergies and allowing for a meaningful analysis of the effect of birch pollen SLIT on BPFA. Furthermore, this study combined patient-reported outcomes measured with validated instruments, immunologic markers, and challenge results, providing a comprehensive and holistic assessment of treatment impact. These robust and multifaceted approaches lay important groundwork for future research and hold promise for advancing effective therapies for BPFA patients.

Conclusion

In conclusion, this analysis of the 1 year results of an ongoing three-year daily practice study demonstrates that birch pollen SLIT treatment leads to meaningful improvements in HRQL and clinical outcomes in patients with BPFA. The comprehensive assessment, integrating clinical, immunologic, and patient-reported measures, provides early evidence of the therapy's potential benefits. These findings support continued investigation of birch pollen SLIT as a promising intervention for BPFA and highlight the potential to better identify patients most likely to benefit, thereby enabling more personalized and effective management of (birch pollen-related) food allergy.

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SUPPLEMENTAL

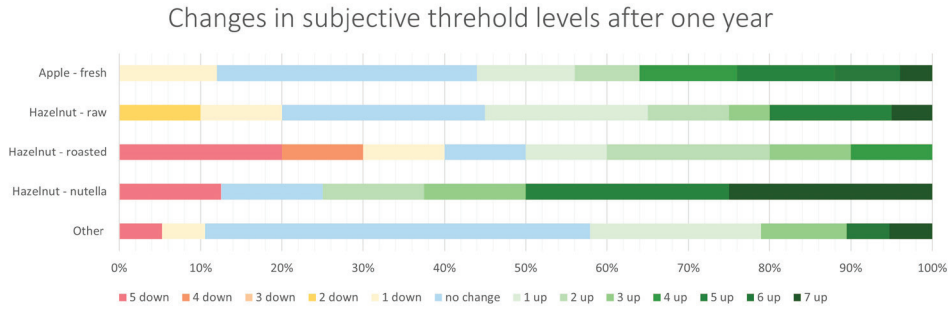


Figure S1. Changes in subjective threshold levels after one year

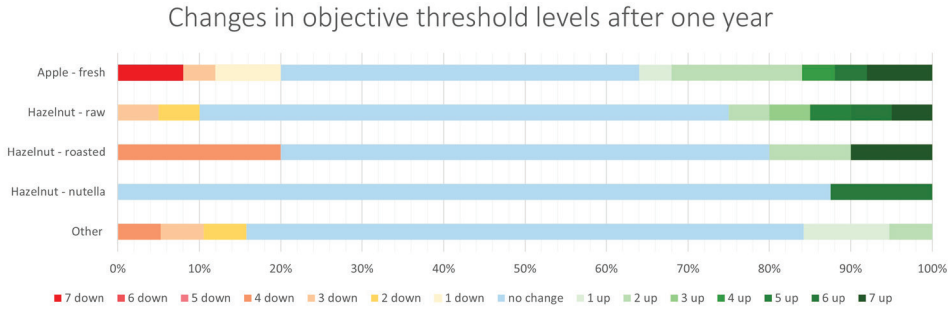


Figure S2. Changes in objective threshold levels after one year

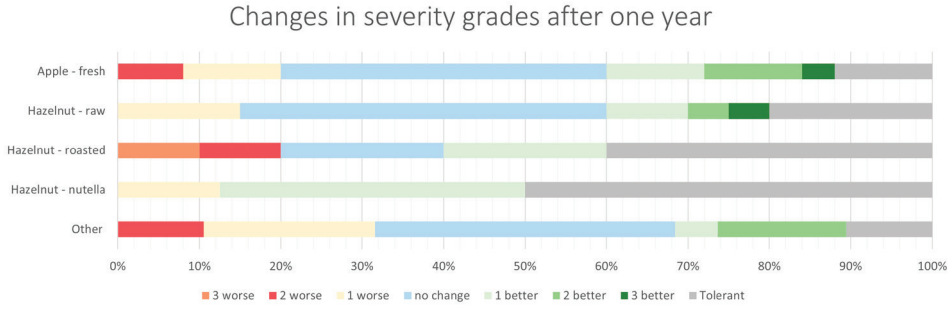


Figure S3. Changes in oFASS-5 severity steps after one year

Table S1. Median wheal sizes with interquartile ranges at baseline and 1 year

	Wheal size in mm; baseline		Wheal size in mm; 1 year		
Allergen	**Median**	**(IQR)**	**Median**	**(IQR)**	**p-value**
Birch	7.5	(6-9.5)	5.5	(5.5-4.25)	**<0.001**
Almond	4.5	(3-5.5)	3.0	(3-2.5)	**0.002**
Hazelnut	5.5	(4-7.5)	5.0	(5-4)	0.083
Pecan nut	0.0	(0-0)	0.0	(0-0)	0.410
Peanut	3.0	(2-4.5)	3.0	(3-1)	0.618
Celery	0.0	(0-2.5)	2.0	(2-0)	0.204
Soy bean	0.0	(0-3)	0.0	(0-0)	0.241
Walnut	2.0	(0-3)	0.0	(0-0)	**0.007**
Carrot	2.5	(0-4)	2.0	(2-0)	**0.026**
Navy bean	2.0	(0-4)	2.0	(2-0)	0.146
Apple	6.3	(3.3-9.6)	4.5	(4.5-3.5)	0.446
Banana	0.0	(0-0)	0.0	(0-0)	0.503
Tomato	3.3	(0-4.5)	2.5	(2.5-0)	0.103
Soy milk	6.0	(3-8)	4.8	(4.8-2.8)	0.362
Tempeh	2.0	(1-2.5)	0.0	(0-0)	0.102
Tofu	3.0	(2.3-5.8)	3.3	(3.3-2)	1.00
Cherry	6.0	(5.5-.)	9.5	(9.5-6.5)	0.18
String bean	3.0	(1-4.75)	2.0	(2-1)	0.668
Kiwi	5.5	(3.8-7.8)	4.0	(4-2.3)	0.568
Bell pepper	2.5	(1.3-4.8)	2.0	(2-0)	0.694

P-values < 0.05 are highlighted in bold

12

General discussion

This thesis contributes to our current understanding of patient characterization and management strategies for food allergy. Here, we will highlight its main findings. The general discussion is divided into four parts: Part I addresses implications for labeling and dietary management; Part II highlights distinct sensitization patterns per region; Part III focuses on improvements in food allergy diagnostics; and Part IV explores the relevance, clinical impact, and treatment of birch pollen-related food allergy (BPFA). The main results and conclusions addressed in each part are as follows:

PART I: TOWARDS BETTER FOOD ALLERGY MANAGEMENT: EXPANDING MANDATORY LABELLING AND PROCESSING-BASED DIETARY RECOMMENDATIONS

- Almost 200 foods can cause a food allergy
- Seeds most often cause severe reactions, despite not being subject to mandatory labelling with the exception of sesame and mustard seeds
- Patients, particularly those with birch pollen-related food allergy, frequently report symptoms after consuming raw hazelnut and soy dairy, while often tolerating the same foods in (more) processed forms

PART II: REGIONAL DIFFERENCES IN ROUTES AND PATTERNS OF (CROSS)SENSITIZATION

- In The Netherlands, sensitization to the PR-10 allergens Pru du 1 (almond) and Pru p 1 (peach) is common due to high birch pollen exposure. However, sensitization to these allergens is also quite common in Portugal and Japan, respectively, despite low birch pollen exposure
- In the Netherlands, sensitization to lipid transfer protein (LTP) Pru du 3 (almond) is far less strongly correlated with Pru p 3 (peach) than in Portugal, suggesting that a primary sensitizer other than peach may be involved outside the Mediterranean area
- In Japan, the gibberellin-regulated protein Pru p 7 (peach) was the predominant allergen detected among peach-allergic patients, whereas in Europe it was rarely detected.

PART III: IMPROVING DIAGNOSTICS OF FOOD ALLERGY

- The 2S albumin seed storage proteins of cashew nut show high diagnostic accuracy, whereas the 2S albumin of almond is not associated with almond allergy
- The gibberellin-regulated protein (GRP) Pru p 7 was the only peach allergen positively associated with severe peach allergy in both Japan and Europe, despite the low sensitization rate in Europe
- A sensor continuously monitoring heart rate, systolic blood pressure, diastolic blood pressure, QT interval, respiratory rate, oxygen saturation, and temperature has the potential to predict a food allergic reaction earlier than an OFC
- Home-based oral food challenges are safe and therefore have the potential to reduce the number of oral food challenges

PART IV: BIRCH POLLEN-RELATED FOOD ALLERGY MATTERS

- Birch pollen-related food allergy (BPFA) symptoms are generally mild to moderate, but severe in 12.9% of patients and these severe reactions are often caused by soymilk, nuts (e.g. raw hazelnut), and tropical fruits (e.g. lychee)
- After one year of treatment with birch pollen sublingual immunotherapy (SLIT), 50.0% of patients with BPFA experienced improvement in health-related quality of life (HRQL), and 66.7% of patients experienced clinical improvement

A detailed discussion of these main findings follows below, outlining their (clinical) implications.

PART I: TOWARDS BETTER FOOD ALLERGY MANAGEMENT: EXPANDING MANDATORY LABELLING AND PROCESSING-BASED DIETARY RECOMMENDATIONS

Pine nut (and sunflower seed) should be considered for inclusion on the precautionary allergen labeling list

In **Chapter 2**, we demonstrated in over a thousand patients that symptoms to almost 200 foods were reported. However, 80% of these symptoms were caused by just 30 of these foods of which pine nut most frequently causes severe symptoms. Despite this, it is not labelled.

Currently, 14 foods (or food groups) are subject to precautionary allergen labeling (PAL) in Europe. The purpose of PAL is to inform and protect allergic consumers.[1] The selection

of foods requiring labeling is based on three criteria: 1) prevalence, supported by studies from different geographic regions; 2) potency, defined by the food's ability to cause allergic reactions based on the eliciting dose and the proportion of individuals who react at each dose; and 3) severity, characterized by the food accounting for at least 5% of all anaphylactic reactions presenting to emergency services in three or more geographic regions.[2] Therefore, one would expect that foods causing frequent and severe reactions are included in the PAL list. Interestingly, although approximately half of the patients who reported symptoms to pine nut and sunflower seed experienced severe reactions, and these allergens caused reactions in 5.3% and 2.2% of patients respectively, they are not labelled (**Chapter 2**).[1] Therefore, we propose that pine nut be considered on the list of ingredients requiring mandatory allergen labeling, and that sunflower seed be considered on the list of emerging food allergens (EFA) (also called 'watchlist'). According to the European Anaphylaxis Registry, seeds are among the leading causes of anaphylaxis, with sesame being the most frequently implicated, followed by pine nut, and sunflower seed.[3] Seeds, including pine nut, are responsible for 4.3% of all food-induced anaphylactic reactions—slightly below the 5% threshold.[2,3] However, celery, which is currently on the mandatory labeling list, accounts for only 2.1% of anaphylactic cases.[3] Furthermore, they also demonstrated that over half of the anaphylactic reactions caused by seeds were triggered by an amount equivalent to a teaspoon or less and that this is considerably lower than the amounts associated with reactions to cow's milk, shellfish, wheat, soya, fish or celery. A recent study also proposed including pine nut on the PAL list, not only due to its frequency and severity, but also because of an increase in anaphylactic reactions to pine nut between 2002 and 2023.[4] Given that pine nut (and sunflower seed) cause severe symptoms in a substantial number of patients with a rising trend in anaphylactic reactions, often at relatively low eliciting doses, and that they are frequently hidden ingredients in processed foods, these seeds should also be considered on the list of ingredients requiring mandatory allergen labeling.

Food processing important in personalized dietary advice

In **Chapter 4, 8, and 9,** we demonstrate that food allergic patients (in particular those with BPFA) more frequently report symptoms after consuming raw or less processed foods, while often tolerating the same food in more processed forms: all patients who experienced symptoms to raw almond tolerated roasted almond (**Chapter 4**), over half of the patients with symptoms to soy milk did not report symptoms to other more processed soy products (**Chapter 8**), and one third of patients who experienced symptoms to raw hazelnut tolerated roasted hazelnut or Nutella (**Chapter 9**As most of the patients had BPFA, the difference in reactivity may for a large part be explained by the heat lability of PR-10 allergens. A review showed that thermal processing may reduce the allergenicity of PR-10 proteins in hazelnut and almond, while lipid transfer proteins (LTP) and seed storage proteins (SSP) in hazelnut and almond appeared very stable.[5] The amount of PR-10 proteins in soybean can also be reduced by heating.[6] Besides heating, other types

of processing could also affect the allergenicity,[7,8] as well as the matrix containing the food.[79] So, less foods are a higher risk for allergic patients, while processed foods may be tolerated by (part of the) patients only recognizing the PR-10 proteins.[5] To provide patients with the best dietary advice, clinicians should perform a thorough history including reactivity to raw/unprocessed and processed food.[5] When patients reacts only to raw/unprocessed, but not to processed food, we recommend no dietary restrictions regarding the processed food. However, when patients react to processed food, without information on the unprocessed food, we recommend performing oral food challenges (OFC) from most processed to unprocessed. For example, in a hazelnut OFC, the challenge can start with hazelnut paste. If tolerated, this can be followed by roasted hazelnut, and finally raw hazelnut if symptoms permit. This approach enables healthcare providers to offer the most accurate and personalized dietary advice regarding food processing, while helping patients avoid unnecessary dietary restrictions.

PART II: REGIONAL DIFFERENCES EXIST IN ROUTES AND PATTERNS OF (CROSS)SENSITIZATION

In both **Chapter 3 and 5**, we observed geographical differences in sensitization patterns, which were consistent with earlier reports: sensitization to PR-10 was dominant in Northern/Central Europe, whereas sensitization to LTP was more prevalent in Southern Europe.[10–13] Interestingly, in addition to these findings, we found that despite birch pollen exposure being uncommon/low in Japan (Sagamihara) and Portugal (Coimbra), sensitization to PR-10 in peach (Pru p 1) and almond (Pru du 1) was quite common in Japan and Portugal, respectively. It is well established that birch pollen Bet v 1 is the primary sensitizer for PR-10 components in food in Northern/Central Europe. Yet, since birch pollen exposure is low in Japan and Portugal, other tree pollen – such as beech in Japan[14] and oak in Portugal[15] – may be responsible for primary sensitization. One study reported that in Central Spain, a birch-free Mediterranean area, 60.5% of patients with pollen food allergy syndrome were sensitized to PR-10 in oak (Que i 1).[16] Furthermore, a small inhibition study showed that in birch free areas (southern Italy), where allergic patients are primarily exposed to *Fagales* pollen other than birch, patients reacted stronger to PR-10 proteins from hornbeam (Car b 1) and oak (Que a 1) than from birch (Bet v 1).[17] This suggest that hornbeam and oak might be the primary sensitizer in these areas.

In Portugal, we observed a strong correlation between sensitization to LTP in almond (Pru du 3) and peach (Pru p 3) in patients, with responses to Pru p 3 being higher (**Chapter 5**). This pattern is consistent with peach typically acting as the primary sensitizer. However, in the Netherlands, no such correlation was observed, suggesting that in non-Mediterranean regions, Pru p 3 is not the primary LTP sensitizer. Instead, mugwort[18–20]

and plane tree[19,20] are hypothesized as primary LTP sensitizer. This may also apply to the Dutch population.

We also observed that peach gibberellin-regulated protein (GRP) Pru p 7 was the most dominant allergen in Japan (**Chapter 3**), with the primary sensitizer being pollen of *Cupressaceae* trees as Japanese cedar[21] (and cypress[22]). In contrast, Pru p 7 was barely recognized by patients in the European centers, likely due to minimal exposure to Japanese cedar pollen and low exposure to cypress pollen in the participating clinical centers in Europe which did not include areas with high cypress pollen exposure. In the Mediterranean region, sensitization to Cupressaceae pollen has increased in recent decades, and with global warming, these species are expected to expand northward, potentially leading to increased sensitization in broader populations.[23] Due to the potentially increased exposure and cross-reactivity between Cupressaceae pollen and peach, we expect more patients to become sensitized to Pru p 7, which may therefore become more clinically relevant in Europe.[22]

These findings suggest that changes in regional environmental factors, such as pollen exposure, should be considered in allergy diagnostics, and that the common sensitizers for PR-10 (birch pollen) and LTP (peach) components may differ per geographic region. Further research is needed to better understand the influence of regional pollen exposure on sensitization profiles.

PART III: IMPROVING DIAGNOSTICS OF FOOD ALLERGY

Seed storage proteins are the best predictors of food allergy in adults but only for selected foods

Our findings indicate that seed storage proteins (SSPs), particularly 2S albumins, have high diagnostic value for most tree nuts in adults, but appear to play no role in the diagnosis of almond allergy. In **Chapter 6**, we demonstrated that sIgE to Ana o 3, the 2S albumin of cashew, was slightly superior to sIgE to cashew extract in predicting cashew allergy in adults (AUC 0.95 *vs* 0.90 in our data, respectively). This is comparable with previous findings in peanut allergic adults, where we observed that both sIgE to Ara h 2 and 6, 2S albumins of peanut, were the best predictors for a peanut allergy (AUC both 0.85 *vs* 0.77 for extract).[24] The hazelnut 2S albumin Cor a 14 performs slightly better than the extract in predicting hazelnut allergy in adults (AUC 0.53-0.70[25–27] *vs.* 0.53-0.54[25,26], respectively). For walnut allergy prediction in adults, the 2S albumin Jug r 1 demonstrated the highest discriminative ability among walnut components, but did not have additional value compared to sIgE to walnut extract or SPT with commercial walnut extract (AUC all around 0.78).[28,29]

In contrast, sensitization to 2S albumin in almond allergic adults was virtually absent (**Chapter 4**). The reason for this remains unclear, but it may be related to the fact that almond is the seed of a stone fruit rather than a true nut, which could influence its allergen profile. In addition, it could also be that the relevance of 2S albumins differ among tree nuts and that the 2S albumin for almond is clinically less relevant in adults.

Our findings indicate that 2S albumins are not always the best predictors of food allergy in adults, with their diagnostic value varying by food. The highest diagnostic accuracy was observed for the 2S albumins in cashew (Ana o 3) and peanut (Ara h 2 and 6).The hazelnut 2S albumin Cor a 14 demonstrated only moderate diagnostic value, marginally outperforming the extract. Walnut Jug r 1 showed comparable performance to extract-based diagnostics, and the diagnostic value of the almond 2S albumin was low. Therefore, we recommend routinely testing sIgE to Ana o 3, Ara h 2, and Cor a 14, in addition to the extract, for patients suspected of cashew, peanut, or hazelnut allergy, respectively. When testing for sIgE to allergen components, simultaneously testing for sIgE to the respective extract is useful to capture broader sensitization and prevent misdiagnosis.[30] For walnut, clinicians may select either sIgE to Jug r 1 or the extract for allergy testing. For almond, we recommend using extract-based testing, and highlight the need for further studies on the diagnostic value of the 7S and 11S storage proteins. Furthermore, the basophil activation test (BAT) could be used to support the diagnosis further.[30]

Peach Pru p 7 is an emerging allergen for peach allergy diagnostics

The gibberellin-regulated protein (GRP) Pru p 7 is the only peach allergen positively associated with severe peach allergy in both Japan and Europe, despite the low sensitization rate in Europe (**Chapter 3**). GRPs are heat-stable and resistant to digestive enzymes, which may explain their relation to severe allergic reactions.[31] Peach Pru p 7 was the first GRP allergen to be described in 2013.[32] Since then, additional GRP allergens have been identified in various foods, including Japanese apricot (Pru m 7), sweet cherry (Pru av 7), orange (Cit s 7), pomegranate (Pun g 7), bell pepper (Cap a 7), and strawberry (Fra a GRP). GRPs have also been described in pollen, although currently restricted to the *Cupressaceae* family, including common cypress (Cup s 7), Japanese cedar (Cry j 7), and mountain cedar (Jun a 7).[33] IgE cross-reactivities among GRP's has been documented and contributes to pollen-food allergy syndrome.[34] As described in Part II, due to cross-reactivity between cypress pollen GRP (Cup s 7) and peach Pru p 7 in Europe, combined with climate change, we expect that sensitization to Pru p 7 will become more common in Northern and Central Europe. Given that Pru p 7 is associated with severe peach allergy (**Chapter 3**), an increase in sensitization may lead to more frequent and more severe reactions to peach in these regions, making Pru p 7 more clinically relevant. In light of these developments, especially for patients with severe peach allergy symptoms, we recommend including sIgE testing to Pru p 7 in the clinical assessment of peach-allergic

patients. Further studies are needed to better characterize GRPs and to understand their role in food allergy, cross-reactivity, and disease severity.

Continuous monitoring: a promising supportive diagnostic tool for earlier detection of food allergic symptoms

In **Chapter 7**, we demonstrated that the CPC12S system, a wearable device capable of continuously monitoring multiple physiological parameters, detected clear changes in heart rate, systolic blood pressure, diastolic blood pressure, QT interval, oxygen saturation, respiratory rate, and temperature during the 10 minutes preceding an objective allergic reaction that occurred during OFC. These findings suggest that wearable devices could play a supportive role during OFC, offering potential for earlier recognition and intervention, and possibly prevention of a severe reaction.[35]

However, as shown in **Chapter 7**, a combined predictive model using the physiological parameters mentioned above achieved only 60% accuracy in predicting allergic reactions during the dose-period *preceding* the one leading to the allergic reaction. The results were somewhat unstable, likely due to the relatively small sample size, highlighting the need for further research and model refinement. The accuracy may also be improved by combining the data of the continuously monitored physiological parameters with clinical characteristics and immunologic markers. This approach is supported by findings in **Chapter 3**, where the highest diagnostic value for prediction of severity was achieved through the combination of clinical characteristics like age of onset and immunologic markers like IgE to peach GRP Pru p 7, instead of using only clinical characteristics or immunologic markers. This has also been shown for hazelnut[27], walnut[12], and peanut[36]. Furthermore, although the CPC12S system is currently among the most advanced wearable devices for continuous monitoring of multiple physiological parameters, the accuracy of blood pressure is low.[37] As changes in blood pressure are important indicators of allergic reactions,[38,39] improving the accuracy of blood pressure measurement may also improve the overall predictive value. Lastly, the current analyses could not determine which physiological parameters were most relevant in triggering an allergic reaction. Gaining better insight into the most relevant (combination of) parameter(s) will be key to improving prediction and clinical usefulness.

In the future, wearable devices could offer valuable opportunities for home monitoring in the management of food-allergic patients. However, before the CPC12S system can be implemented in a home setting, both the device itself and our understanding of the relationship between physiological parameters and allergic reactions must be further improved. Current wearable devices for home monitoring, such as smartwatches, are often not validated for comprehensive clinical use.[40] Furthermore, of the physiological parameters that could change during an allergic reaction, most devices only measure heart rate reliably. Blood pressure is difficult to measure accurately without a cuff and

therefore, most wearables either do not offer it, or rely on unvalidated optical methods. Oxygen saturation is available in many wearables now, but is not very accurate, especially during motion or poor skin contact.[41] One study that compared measurements of a smart watch with hospital equipment even showed poor correlations across all domains evaluated including heart rate, oxygen saturation, and respiration with the highest correlation coefficients of 0.62 but with most below 0.5.[42]

To conclude, wearable devices have a high potential for improving food allergy diagnosis, but before such systems can be reliably implemented in clinical and home settings, both technological improvements and further clinical validation are essential.

Home-based oral food challenges: reduce waiting lists for oral food challenges

In **Chapter 9**, we showed that home-based OFCs (HOFC) can be performed safely when patients are well-informed and carefully selected. As such, HOFC may potentially serve as a valuable diagnostic tool to support the diagnosis of food allergy. As the diagnostic accuracy of current sensitization tests is limited, oral food challenges (OFCs) are still the gold standard for diagnosing food allergy.[30] However, OFCs are costly, burdensome, resource-intensive and often there is a long waiting list. During the time between the onset of symptoms and the scheduled OFC, patients are typically advised to avoid the suspected food(s) by their healthcare providers. Furthermore, OFCs may be negative, indicating that prior food avoidance was unnecessary. Positive OFC rates vary widely across studies and populations. According to the EAACI guideline, fewer than 50% of patients undergoing OFC are allergic[30], whereas in more selected patients, positivity rates can reach up to 100%.[43–45] Food avoidance carries several risks, including nutritional deficiencies and even the development of IgE-mediated food allergy.[46] Nachson et al. showed that food allergies can develop after just seven days of avoiding a previously tolerated food, with a median avoidance period of 45 weeks[47] Furthermore, atopic patients who have a strong predisposition for IgE production may be particularly susceptible to develop food allergies following avoidance of previously tolerated foods.[47,48] Early evaluation of the suspected food may be protective against the development of food allergy. With appropriate guidance, HOFC can serve as a safe and practical approach to facilitate such an early evaluation. To ensure safety, HOFCs should follow a structured approach: 1) select only patients who reported mild symptoms in their clinical history, 2) provide clear written instructions for performing the HOFC, 3) start the HOFC with low doses, 4) explain criteria for stopping the HOFC, 5) supply an emergency action plan, and 6) ensure easy and direct contact with a healthcare provider. Furthermore, performing HOFC in patients with mild symptoms may reduce delays in performing OFC in patients with severe symptoms.

To date, no other studies have investigated HOFCs. However, in April 2025, the Food Challenge at Home or in Medical Practice (CHOMP) trial was launched.[49] In this trial, adults with a self-reported food allergy and a negative SPT of the implicated food are randomized to perform either a standard OFC or a HOFC. This study aims to determine the safety of HOFC, as well as the feasibility of recruitment and delivery, protocol adherence, health-related quality of life, and food reintroduction outcomes. Although patients in our study were all sensitized, the results of the CHOMP trial may provide valuable insights into the feasibility and safety of HOFCs on a broader scale.

Implementing HOFCs has the potential to provide early diagnosis for patients with mild symptoms and shorten OFC waiting lists for patients with more severe symptoms. This may prevent unnecessary food avoidance, alleviate the burden on healthcare providers, and lower costs.

PART IV: BIRCH POLLEN-RELATED FOOD ALLERGY MATTERS

Bich pollen-related food allergy can be more severe than previously thought

We demonstrated that birch pollen-related food allergy (BPFA) is the most common type of food allergy in the Netherlands (**Chapter 2**). In the same chapter, we showed that 32.7% of patients with PR-10 monosensitization reported severe symptoms. Additionally, **Chapter 9** showed that 12.9% of food challenges in BPFA patients resulted in severe reactions. Despite this, the majority of studies have assumed BPFA as generally mild to moderate, with severe symptoms considered rare.[50–52] However, recent evidence challenges this assumption. [3]A study from Germany highlighted that in a cohort of food anaphylactic patients PR-10 sensitization was identified as the leading protein family.[53] Asero et al. investigated the cause of PR-10-induced systemic reactions and showed that 56% were facilitated by cofactors like proton pomp inhibitor use, fasting, and ingestion of large amounts of unprocessed foods.[54] However, in 44% of reactions no cofactor was identified, underscoring the intrinsic potential of PR-10 proteins to trigger severe symptoms. Collectively, these findings confirm that BPFA, while often mild, can result in severe reactions and should not be underestimated. Healthcare providers should be aware of potential cofactors that may increase the risk of systemic reactions in (BPFA) patients.

In both **Chapter 2 and 9**, severity was assessed using the oFASS severity score, a recently developed and comprehensively validated severity scoring system.[55] While oFASS effectively distinguishes between mild oropharyngeal symptoms and moderate to severe symptoms, it does not distinguish between varying degrees of pulmonary symptoms. Therefore, it classifies mild dyspnea and severe respiratory distress under the same

severity score which may overestimate the clinical severity in some cases. Despite this limitation, all patients classified as severe in **Chapter 9** experienced objective pulmonary symptoms, with nearly half showing a decrease in peak flow. Furthermore, almost half of the challenges were discontinued for safety reasons according to predefined stopping criteria, suggesting that more severe symptoms may have developed if the challenge had been continued. These findings underscore how a severity scoring system can influence interpretation of risk.

The foods that most frequently elicited severe reactions in patients with BPFA were soy milk, hazelnut, walnut, and cherry (**Chapter 9**). Furthermore, also **Chapter 8** showed that of the soy products, soy dairy caused most frequently severe symptoms. Severe reactions to soy dairy have been repeatedly reported in patients with BPFA.[54,56,57] It is hypothesized that these severe reactions were provoked by high PR-10 (Gly m 4) contents.[6,57] Furthermore, in **Chapter 2,** we demonstrated that 20-70% of patients reported severe symptoms for tropical fruits like lychee, papaya, and jackfruit. Although PR-10 components have not yet been identified in all these fruits, it is speculated that the reactions were related to BPFA.[54,58]

We expect BPFA to become a bigger health issue in the future, driven by both environmental and lifestyle changes.[59] As demonstrated in **Chapter 2**, plant-based foods were the most common cause of food allergy. With the ongoing shift towards a more plant-based diet, we expect an increase in the prevalence of plant-food allergy. Moreover, severe reactions are likely to increase as consumers more frequently eat exotic tropical fruits like lychee[3,60] and high-protein plant-based products like soy.[54,61] However, for common plant foods such as apple, an increase in anaphylactic reactions has already been observed between 2000 and 2023.[4] Climate change may also affect BPFA by altering pollen exposure and sensitization patterns in various ways, including shifting and lengthening of pollen season, altering pollen concentration and allergenicity, and redistributing allergic plants geographically.[62] These changes could potentially increase the risk and severity of BPFA.

Towards a cure for birch pollen-related food allergy?

In **Chapter 10**, we concluded that there was insufficient evidence to draw definitive conclusions regarding the effectiveness of birch pollen AIT in treating BPFA. To address this evidence gap, we initiated a study investigating the effect of birch pollen sublingual immunotherapy (SLIT) on BPFA (**Chapter 11**). We observed that after one year of birch pollen SLIT, 50.0% of the patients with BPFA experienced an improvement in their HRQL, and 66.7% exhibited a clinical response. These results make it unlikely that the observed effects were due to a placebo response, which was defined to result in an improvement of at most 20%. Several factors may explain why the results were more positive in **Chapter 11** than in **Chapter 10**. In **Chapter 10**, which presented a systematic review of ten articles, we focused mainly on the three studies with the lowest risk of bias. These

three studies all investigated subcutaneous immunotherapy (SCIT), not SLIT. It is possible that SLIT exerts more favorable effects due to its local immunomodulatory actions at the site of allergen administration, i.e. the oral mucosa, where the allergic immune response is initially triggered. SLIT takes advantages of the tolerogenic environment of the oral mucosa to promote tolerance to the allergen.[63] It has been suggested that oral mucosal Langerhans Cells that had taken up birch pollen, produce regulatory cytokines such as IL-10 and transforming growth factor (TGF)-β1, both known to contribute to a tolerogenic microenvironment.[63,64] Additionally, SLIT may stimulate the production of allergen-specific IgA, which acts as a first line of defense at mucosal barriers and could block the binding of allergen-IgE complexes at mucosal surfaces.[63] Given that SLIT is administered at the mucosal surface, and these above mentioned processes are specific for the mucosa, it is possible that such localized immune mechanisms play a central role in the clinical efficacy observed with this treatment.

Beyond the route of administration, better **patient profiling** may also contribute to the positive effect. In **Chapter 11** careful molecular characterization supported the diagnosis BPFA. Among the three low risk of bias studies included in **Chapter 10**, one study showed no improvement on symptom severity and even a negative effect on eliciting dose following birch pollen SCIT. This study investigated birch pollen–related hazelnut allergy but patients were less well molecularly characterized: only sensitization to Cor a 1 and Cor a 8 were measured, but not Cor a 9 and Cor a 14.[65] It is possible that part of the participants were co-sensitized to Cor a 9 and 14, meaning they were not purely PR-10–sensitized BPFA patients, which could have confounded the observed effects of birch pollen AIT. In contrast, in **Chapter 11**, we included only patients sensitized to PR-10 components and *not* to non-PR-10 components, based on the available components, to ensure the most homogeneous BPFA population possible.

Only one other study evaluated birch pollen SLIT in apple allergic patients, using the same drug as described in **Chapter 11**.[66] After 6.5-9.5 months of treatment, 75% of patients in the SLIT group and 68% of patients in the placebo group reached the highest tolerated dose of apple. However, this study was conducted in a subgroup of participants from a phase III double-blind, placebo-controlled trial who were not preselected for BPFA, and no OFC was performed before the start of treatment. As a result, it is possible that many patients already tolerated the highest dose of apple before treatment even began. This limits the interpretability of the findings and makes them difficult to compare directly with our study, in which challenges were performed both before and after one year of treatment, and only patients who were allergic to at least one BPFA-related food were included (**Chapter 11**).

To conclude, the positive effect of birch pollen SLIT observed in **Chapter 11**, may be due to the use of SLIT instead of SCIT in combination with the careful selection of patients.

Other treatment approaches beyond birch pollen AIT have also been explored in birch pollen-related apple allergic patients. For example, Kinaciyan et al. investigated the effect of SLIT with either apple PR-10 (Mal d 1), birch PR-10 (Bet v 1), or placebo on apple allergy after four months of treatment.[67] Interestingly, only the Mal d 1 SLIT group demonstrated significant clinical improvement, accompanied by an increase in IgG4 to Mal d 1. In contrast, the Bet v 1 SLIT group did not show clinical improvement but was the only group to exhibit increases in all measured immunological markers (IgG4 and IgE to both Mal d 1 and Bet v 1). No significant clinical or immunological changes were observed in the placebo group. This findings suggest that, clinically, Mal d 1 SLIT was more effective, whereas immunologically, more changes were observed using Bet v 1 SLIT. Another study of Kopac et al. demonstrated that after eight months of oral immunotherapy with apple, 13/27 patients were able to tolerate a whole apple, compared to 0/13 patients in the untreated group ($p = 0.0001$).[68] However, there we no significant differences in immunologic changes between the treatment and control group. Together, these findings suggest that clinical tolerance in birch pollen-related apple allergy can be achieved by allergen specific immunotherapy and dietary exposure, but the limited/absent immunologic changes suggest that the induced tolerance may be only transient.

The positive findings from **Chapter 11** demonstrate the potential of birch pollen SLIT as a treatment for BPFA. Unlike food-specific immunotherapy, it is applicable to the whole range of BPFA-related foods. In addition, it simultaneously treats birch pollen allergy and may therefore help prevent further expansion of BPFA symptoms over time. The administration as a sublingual tablet, rather than subcutaneous injections, also makes it an easy to use treatment. The current data reflect only on the one-year results, longer term outcomes remain to be awaited.

FOOD FOR THOUGHT

Future considerations in food allergy should integrate both patient characterization and management strategies to improve diagnosis, prevention, and treatment of patients with food allergies. Adding pine nut to the list of mandatory labelling allergens should be considered, as they cause one of the most frequent and severe food allergic reactions. Furthermore, standardized dietary advice regarding food processing is needed, especially for patients with BPFA. Home-based oral food challenges could complement this dietary advice and may provide early diagnosis in patients with mild symptoms, while alleviating the burden on healthcare providers and reducing waiting list for OFC for patients with severe symptoms. Additionally, wearable sensors may eventually support OFC by continuously monitoring physiological parameters that could help predict a food allergic reaction, with further developments potentially enabling their use in home monitoring. Bich pollen-related food allergy (BPFA) may become a bigger health issue due to lifestyle

and environmental changes, so health-care providers should not underestimate its clinical impact. Nevertheless, birch pollen SLIT is a promising treatment for patients with BPFA.

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English summary | Nederlandse samenvatting

ENGLISH SUMMARY

Background food allergy

Food allergy has a major global health impact. Among adults, the prevalence based on self-reported symptoms varies between 2% and 37% across Europe, whereas the true prevalence, as confirmed by an oral food challenge, ranges between 0.2% and 4.1%.

A food allergy is an abnormal immune response to specific proteins in food. Before someone becomes allergic, they must first become sensitized. This means that the immune system recognizes a specific food protein and produces specific antibodies against it, known as IgE antibodies. However, not everyone who is sensitized will actually develop a food allergy.

In principle, any food can cause an allergic reaction. Previous research has mainly focused on the eight most common allergenic foods: cow's milk, hen's eggs, wheat, soy, peanut, tree nut, fish and shellfish. However, little is known about the frequency and severity of allergies to other foods. Our research has demonstrated that patients can be allergic to as many as 200 different foods in daily clinical practice, although the majority of reactions are caused by approximately 30 foods (**Chapter 2**). Notably, allergic reactions to fruit are common, with apple and kiwi being among the most frequently reported, followed by tree nut and peanut.

The symptoms of a food allergy can range from mild reactions, such as itching in the mouth, to severe and potentially life-threatening reactions, including drop of blood pressure and loss of consciousness. Our study showed that seeds, including sesame, sunflower and pine nut, most frequently cause severe symptoms. Additionally, exotic fruit such as lychee and papaya were regularly associated with severe reactions (**Chapter 2**).

These findings demonstrate that a much broader range of foods than has been investigated to date can cause allergic reactions and that some of these foods are frequently associated with severe symptoms.

Certain specific allergenic proteins are important predictors of the presence and severity of food allergy

When there is a suspicion of food allergy based on the clinical history, it is common practice to assess whether patients have specific IgE (sIgE) to the foods that cause their symptoms (sensitization). This can be measured by skin prick tests and blood tests.

Foods contain allergenic proteins that often share a structural similarity because they belong to the same protein families. IgE antibodies directed to certain protein families appear to be better predictors of food allergy than those directed to whole food extracts.

For example, those directed to so-called 2S albumins, storage proteins abundantly present in legumes, nuts and seeds, may be more accurate in confirming or excluding allergy than standard extract-based testing. In adults with suspected cashew allergy, we demonstrated that sIgE to Ana o 3 (the 2S albumin protein in cashew) correctly classified 72% of patients as allergic or non-allergic (**Chapter 6**). In contrast, sIgE against cashew extract correctly classified only 49% of patients.

In light of this favorable outcome, we also explored this approach in adults with suspected almond allergy (**Chapter 5**). Surprisingly, 2S albumin of almond was not found to play a similar role as reported for peanut, several tree nuts and sesame seed. Consequently, this protein cannot be used as a predictor of almond allergy. Also none of the other almond proteins and extract tested proved to be a reliable predictor either (**Chapter 4 and 5**).

In addition to establishing the diagnosis, IgE directed to specific proteins may also help to assess the potential severity of a food allergy. For example, sIgE to the peach lipid transfer protein Pru p 3 has been recognized as a risk factor for severe symptoms in patients with peach allergy. We have shown that the presence of sIgE to the more recently identified peach allergen Pru p 7 is a risk factor for severe peach allergy in adults in both Europe and, even more clearly, in Japan (**Chapter 3**).

Regional differences in patterns of allergen recognition

It is well established that sensitization to proteins from the lipid transfer protein (LTP) family is more common in the Mediterranean region, whereas PR-10 proteins predominate in Northern European countries, mainly due to cross-reactivity with highly prevalent birch pollen. In this thesis, we confirmed these patterns for peach (**Chapter 3**) and almond (**Chapter 5**). In addition, we extended these findings.

Despite the low concentration of birch pollen in the Mediterranean region and Japan, patients in these areas were frequently sensitized to PR-10 proteins (**Chapter 3**). This suggests that cross-reactivity also plays a role in these areas, possibly driven by other tree species such as oak.

Peach is often considered the primary sensitizing source for lipid transfer proteins. However, this is not what we observed in the Netherlands (**Chapter 5**). Other food sources, or perhaps pollen such as mugwort or plane tree pollen, may act as the initial sensitizing trigger in the Netherlands.

These findings demonstrate that patterns of allergen recognition differ between Northern and Southern Europe. Although the same allergens are recognized, albeit in different proportions, is likely due to different origins of sensitization.

Improving the diagnosis of food allergy by monitoring or conducting home-based oral food challenges

The gold standard for diagnosing a food allergy is an oral food challenge, during which the patient is given gradually increasing amounts of the suspected food in a hospital setting. A food challenge provide valuable information about the presence of food allergy, potential severity of a reaction and the dose at which symptoms occur.

Food challenge outcomes vary in severity and severe reactions can occur. We investigated whether continuous monitoring during an oral food challenge could predict a severe reaction before objective allergic symptoms occurred using the following key vital parameters: heart rate, blood pressure, QT interval, respiratory rate and body temperature (**Chapter 7**). Our study demonstrated that a combination of these parameters could predict an allergic reaction with approximately 60% accuracy before it became clinically apparent. If these results can be confirmed, oral food challenges could potentially be discontinued earlier, thereby reducing the frequency or severity of serious reactions.

An oral food challenge is labor- and time-intensive, and is therefore associated with substantial costs. In addition, high demand and limited capacity often result in long waiting lists. Our results show that, for certain patients, a home-based oral food challenge could be a suitable alternative (**Chapter 9**). Based on careful patient selection and with thorough instruction, we determined whether the test could be safely performed at home. The criteria were as follows: 1) Only patients with a history of mild symptoms were selected. 2) These patients received clear verbal and written instructions outlining the procedure and how to act in case of a reaction. 3) The home-based oral food challenge was always initiated with the lowest dose. 4) Clear stop criteria were defined in advance and provided in writing. 5) Emergency medication was supplied and 6) direct, easily accessible contact with hospital staff was ensured throughout the process.

Our results demonstrate that home-based oral food challenges performed in accordance with the above-mentioned criteria was safe for all patients who underwent the procedure. Future research should focus on optimizing the implementation of home-based oral food challenges and evaluating their impact on reducing workload and healthcare costs.

Birch pollen-related food allergy: underestimated impact and potential for treatment

Birch pollen-related food allergy is the most common food allergy in Northern and Central Europe. It affects individuals who are already allergic to birch pollen. The PR-10 protein found in birch pollen shows strong similarity with related proteins found in many different foods, which can lead to cross-reactivity. Consequently, patients often exhibit

allergies to multiple foods simultaneously. We demonstrated that this can involve many foods, in our study up to 16 (**Chapter 9).**

The foods that most frequently cause symptoms are fruits such as apple and peach, nuts such as hazelnut and walnut, legumes such as soy and peanut, and vegetables such as carrot and celery (**Chapter 9**).

The symptoms of birch pollen-related food allergy are generally mild, such as oral itching. However, this is not always the case. This thesis found that 13% of birch pollen allergic patients undergoing an oral food challenge experienced severe reactions (**Chapter 9**). Specifically, patients who underwent a food challenge for soy milk in our study experienced severe symptoms. Fresh cherry and raw walnut were the next most frequent causes of severe reactions (both 33%). In absolute numbers, raw hazelnut most frequently caused severe symptoms.

Patients with a birch pollen-related food allergy experience a reduction in quality of life (**Chapter 9**). Mostly because they are afraid of having a food allergic reaction, they constantly monitor their diet and find it difficult to maintain a healthy lifestyle. Furthermore, the foods they are allergic to are essential components of a nutritious diet.

Currently, there is no treatment available for patients with a birch pollen-related food allergy. As birch pollen-related food allergy is associated with birch pollen allergy, which can be treated with birch pollen immunotherapy, we conducted a literature review to investigate whether this treatment could also be effective for birch pollen-related food allergy (**Chapter 10**). The review revealed that few high-quality studies have been conducted and that their results were inconsistent. Therefore, we investigated whether immunotherapy using a sublingual tablet containing birch pollen could be an effective treatment for patients with birch pollen-related food allergy (**Chapter 11**). Already after one year of treatment, half of the patients reported a significant improvement in their food allergy-related quality of life. Additionally, two-third of patients reported a reduction in symptom severity or were able to tolerate a larger amount of the causative food. Given these positive results, patients will be followed for further evaluation.

Personalized dietary advice for food allergy

There is no standard treatment available for patients with food allergy. Therefore, guidance is provided on how to manage allergic reactions using an emergency kit, as well as on how to follow an appropriate diet. As patients with birch pollen-related food allergy often have to avoid many foods or can only tolerate them in a specific processed form, personalized dietary advice is needed. It is known that patients usually do not experience symptoms from processed fruits and vegetables (such as apple sauce), but react primarily to fresh products. However, it is unclear whether this also applies to nuts and soy.

In this thesis, we demonstrate that patients who experienced adverse reactions to raw almond did not react to roasted almond. In such cases, roasted almond does not need to be avoided (**Chapter 4**). Of patients allergic to raw hazelnut, one-third did not report symptoms when consuming roasted hazelnut or hazelnut paste (**Chapter 9**).

For soy, we created a ranking of different products based on the frequency and severity of symptoms they caused. From most to least frequent, the order was: soy dairy, tofu, soybean and tempeh, meat substitute, soy sauce, bread and soy containing biscuit "Biscoff". The order for severity of symptoms was quite similar: soy dairy caused the most severe reactions, followed by tofu, meat substitute, soybean and tempeh, soy sauce, bread and "Biscoff". Additionally, 53% of patients who reported symptoms to soy milk did not experience reactions to other soy products.

The fact that reactions to different processed (soy) products vary in both frequency of causing symptoms and severity of symptoms, and that many patients tolerate certain products, suggests that the diet can be personalized.

New candidates for allergen labelling

To enable individuals with a food allergy to properly follow dietary advice, allergen labelling provides important guidance. Allergen labelling provides important guidance for individuals with a food allergy. In Europe, labelling is currently mandatory for 14 foods or food groups, including tree nut, peanut, cow's milk, soy, hen's egg, shellfish, sesame, fish, cereal, celery, mustard, mollusc, lupin and sulphur dioxide/sulphites.

However, foods that are currently not labelled can still cause frequent and severe reactions. Our research showed that pine nut was responsible for 5% of food-allergic reactions, 51% of which were severe (**Chapter 2**). For sunflower seed, the respective figures were 2% and 43%. Based on these results, it may be advisable to consider labelling pine nut in the future and placing sunflower seed on a 'watch list' for potential future allergen labelling.

Key conclusions of this thesis

- Almost 200 different foods can cause a food allergy
- The 2S albumin protein of cashew is a reliable predictor of cashew allergy, whereas that of almond is not a reliable predictor of almond allergy
- The peach allergen Pru p 7 is the most reliable indicator of severe peach allergy in both Europe and Japan
- Continuous monitoring of heart rate, blood pressure, QT interval, respiratory rate and body temperature shows promising results in predicting allergic reactions and may

enable earlier conclusions from oral food challenges, preventing severe allergic reactions during OFC
- A home-based oral food challenge is safe, provided certain conditions are met
- Birch pollen-related food allergy generally causes mostly mild symptoms, but can lead to severe reactions in more than 10% of patients
- Sublingual immunotherapy with birch pollen may be an effective treatment for patients with birch pollen-related food allergy
- Pine nut and sunflower seed cause frequent and severe reactions, and may therefore be candidates for labeling

NEDERLANDSE SAMENVATTING

Achtergrond voedselallergie

Voedselallergie heeft wereldwijd een grote impact. Bij volwassenen ligt de prevalentie op basis van zelf gerapporteerde klachten in verschillende Europese landen tussen de 2 en 37%, terwijl de werkelijke prevalentie zoals bevestigd met een voedselprovocatie tussen de 0,2 en 4,1% ligt.

Voedselallergie is een abnormale reactie van het immuunsysteem op specifieke eiwitten in voedingsmiddelen. Voordat iemand allergisch wordt, raakt men eerst gesensibiliseerd. Dit betekent dat het immuunsysteem het specifieke voedseleiwit herkent en er specifieke antistoffen tegen aanmaakt, ook wel specifiek IgE (sIgE) genoemd. Echter, niet iedereen die gesensibiliseerd is, ontwikkelt daadwerkelijk een allergie.

In principe kunnen alle voedingsmiddelen een voedselallergie veroorzaken. Eerder onderzoek heeft zich voornamelijk gericht op de acht meest bekende voedingsmiddelen die een voedselallergie veroorzaken, namelijk: koemelk, kippenei, tarwe, soja, pinda, noten, vis en schaal- en schelpdieren. Over de frequentie en ernst van allergieën voor andere voedingsmiddelen is echter weinig bekend. Wij hebben laten zien dat patiënten in de dagelijkse praktijk allergisch kunnen reageren op wel 200 verschillende voedingsmiddelen, waarbij het merendeel van de reacties wordt veroorzaakt door 30 voedingsmiddelen (**Hoofstuk 2**). Opvallend hierbij is dat allergische reacties op fruit frequent voorkomen; appel en kiwi behoren daarbij tot de meest gerapporteerde voedingsmiddelen, gevolgd door noten en pinda. De klachten bij een voedselallergie variëren van milde symptomen, zoals jeuk in de mond, tot ernstige en zelfs potentieel levensbedreigende symptomen, waaronder bewustzijnsverlies. Onze studie liet zien dat zaden en pitten (waaronder sesam, zonnebloempit en pijnboompit) het meest frequent ernstige klachten geven. Verder bleek dat exotisch fruit (zoals lychee en papaja) ook regelmatig ernstige klachten geeft. (**Hoofstuk 2**).

Deze bevindingen illustreren dat een veel breder dan voorheen onderzocht spectrum aan voedingsmiddelen allergische reacties kan veroorzaken, waarbij sommige voedingsmiddelen frequent ernstige klachten geven.

Sommige specifieke eiwitten zijn belangrijk voorspellers van zowel de aanwezigheid als de ernst van een voedselallergie

Als er op basis van het gesprek tussen de arts en patiënt, een verdenking op een voedselallergie bestaat, wordt vaak nagegaan of patiënten specifiek IgE (sIgE) hebben tegen voedingsmiddelen waarvoor ze klachten rapporteren (sensibilisatie). Dit kan gemeten worden middels huidpriktesten en bloedonderzoek.

In verschillende voedingsmiddelen zitten allergene eiwitten die sterk op elkaar lijken omdat ze tot dezelfde eiwitfamilies behoren. IgE antistoffen tegen sommige van dergelijke eiwitgroepen blijken betere voorspellers te zijn voor het al dan niet aanwezig zijn van een allergie dan IgE antistoffen tegen extracten. Zo kunnen zogenaamde 2S-albumine eiwitten in verschillende soorten plantaardige voeding een allergie beter aantonen of uitsluiten dan met de standaardtest (extract). Wij hebben aangetoond dat bij volwassenen met een verdenking op een cashew allergie het sIgE tegen Ana o 3, het 2S-albumine eiwit in cashew, bij 72% van de patiënten correct kon worden aangegeven of ze allergisch waren of niet (**Hoofdstuk 6**). Voor cashew extract bedroeg dit percentage slechts 49%.

Gezien dit gunstige resultaat hebben we dit ook bestudeerd bij volwassenen met een verdenking op een amandel allergie (**Hoofdstuk 5**). Tot onze verrassing bleek dat bijna niemand sIgE had tegen deze 2S-albumine eiwitten. Hierdoor is het dus niet mogelijk om dit eiwit als voorspeller te gebruiken voor een amandel allergie. Helaas bleek ook geen van de andere eiwitten een goede voorspeller (**Hoofdstuk 4 en 5**).

Naast het stellen van de diagnose kan IgE voor specifieke eiwitten ook bijdragen aan het inschatten van de ernst van een voedselallergie. Voor perzikallergie was tot op heden vooral sIgE tegen het lipid transfer protein in perzik, Pru p 3, bekend als een risicofactor voor ernstige symptomen. Wij hebben aangetoond dat aanwezigheid van sIgE tegen het meest recent geïdentificeerde perzik allergeen, Pru p 7, zowel in Europa en zelf nog meer in Japan een risicofactor is voor ernstige perzikallergie bij volwassenen (**Hoofdstuk 3**).

Regionale verschillen in patronen van allergeenherkenning

Het is algemeen bekend dat in het Mediterrane gebied sensibilisatie voor eiwitten uit de lipid transfer protein familie vaker voorkomt, terwijl in Noord-Europese landen vooral de PR-10 eiwitten domineren door kruisreacties met de veelvoorkomende berkenpollen. In dit proefschrift hebben wij deze patronen kunnen bevestigen voor perzik (**Hoofdstuk 3**) en amandel (**Hoofdstuk 5**). Daarnaast hebben wij deze bevindingen kunnen uitbreiden. Ondanks de lage concentratie berkenpollen in het Mediterrane gebied en in Japan, bleek dat patiënten in deze regio's toch regelmatig gesensibiliseerd waren voor PR-10-eiwitten (**Hoofdstuk 3**). Dit suggereert dat ook hier kruisreacties een rol spelen veroorzaakt door andere boomsoorten, zoals bijvoorbeeld eik. Voor lipid transfer protein is perzik vaak de initiële sensibiliserende bron. In Nederland bleek dat niet het geval te zijn (**Hoofdstuk 5**). Mogelijk spelen andere bronnen, zoals bijvoet of plantaan een rol als initiële sensibiliserende factor. Dit laat zien dat patronen van allergeen herkenning verschillen tussen Noord en Zuid Europa. Hoewel dezelfde allergenen worden herkend (in verschillende percentages), kan dit het gevolg zijn van een andere sensibilisatieroute.

Het stellen van de diagnose voedselallergie kan worden verbeterd via monitoring en thuisprovocaties

Om met zekerheid vast te stellen of iemand allergisch is of niet, kan het nodig zijn een voedselprovocatie te verrichten. De patiënt krijgt dan in het ziekenhuis geleidelijk toenemende hoeveelheden van het verdachte voedingsmiddel toegediend. Daarnaast kan een provocatie ook bijdragen aan het verkrijgen van informatie over de mogelijke ernst van een eventuele reactie en bij welke hoeveelheid van het voedingsmiddel klachten te verwachten zijn.

Tijdens een provocatie treedt in sommige gevallen een ernstige reactie op. Wij hebben onderzocht of continue monitoring van belangrijke lichaamsfuncties, namelijk hartslag, bloeddruk, QT-interval, ademhalingsfrequentie en lichaamstemperatuur een ernstige reactie kan voorspellen voordat er objectieve (waarneembare) allergische klachten optreden (**Hoofdstuk 7**). In onze studie konden wij met ongeveer 60% nauwkeurigheid aantonen dat met de combinatie van bovengenoemde lichaamsfuncties het mogelijk is om zo'n allergische reactie te voorspellen voordat deze waarneembaar is. Als de resultaten bevestigd worden, kunnen provocaties mogelijk eerder worden gestaakt, bijvoorbeeld al bij subjectieve klachten. Dit kan ervoor zorgen dat ernstige klachten niet of minder vaak optreden.

Een voedselprovocatietest is arbeids- en tijdsintensief en brengt daardoor aanzienlijke kosten met zich mee. Daarnaast leiden de hoge vraag en beperkte capaciteit vaak tot lange wachtlijsten in het ziekenhuis. Onze resultaten laten zien dat in sommige gevallen een thuis provocatietest een alternatief kan zijn (**Hoofdstuk 9**). Hierbij wordt, op basis van een zorgvuldige selectie en instructie van patiënten, besloten of de test veilig thuis kan worden uitgevoerd. Deze waren als volgt: 1) alleen patiënten met milde klachten werden geselecteerd, 2) duidelijke mondeling en schriftelijke instructies werden gegeven over de uit te voeren stappen en wat te doen bij een reactie, 3) de thuisprovocatie werd altijd gestart met de laagste dosering, 4) duidelijke stopcriteria werden afgesproken en op schift meegegeven, 5) noodmedicatie werd meegegeven en 6) direct en laagdrempelig contact met ziekenhuispersoneel werd gewaarborgd. Onze resultaten laten zien dat thuisprovocaties veilig zijn bij alle patiënten die dit hebben gedaan. Vervolgonderzoek dient zich te richten op de optimale inzet van thuisprovocaties en het effect hiervan op het verlagen van werkdruk en zorgkosten.

Het paraberksyndroom is belangrijker dan gedacht en mogelijk behandelbaar

Het paraberksyndroom is de meest voorkomende voedselallergie in Noord- en Centraal-Europa. Het ontstaat bij patiënten die al allergisch zijn voor berkenpollen. Het eerdergenoemde PR10 eiwit in berkenpollen vertoont sterke gelijkenis met verwante eiwitten in veel verschillende voedingsmiddelen, waardoor kruisallergieën kunnen optreden. Hier-

door zijn patiënten vaak allergisch voor meerdere voedingsmiddelen tegelijk. Wij hebben aangetoond dat dit kan oplopen tot wel 16 verschillende voedingsmiddelen (**Hoofdstuk 9**).

De voedingsmiddelen die het meest frequent klachten veroorzaken zijn fruitsoorten zoals appel en perzik, noten zoals hazelnoot en walnoot, peulvruchten zoals soja en pinda en groenten zoals wortel en selderij (**Hoofdstuk 9**).

De klachten bij het paraberksyndroom zijn over het algemeen mild, zoals jeuk in de mond, maar dit is niet altijd het geval. In dit proefschrift laten we zien dat bij 13% van de patiënten die een voedselprovocatie ondergingen, ernstige klachten optraden (**Hoofdstuk 9**). Alle patiënten die een provocatietest met soja (in de vorm van sojamelk) ondergingen, hadden ernstige klachten. Daarna kwamen verse kers en rauwe walnoot het vaakst voor als oorzaak van ernstige reacties (allebei 33%). In absolute aantallen gaf rauwe hazelnoot het meest frequent ernstige klachten.

Daarnaast ervaren patiënten met het paraberksyndroom een beperking in hun kwaliteit van leven (**Hoofdstuk 9**). Deze verminderde kwaliteit van leven wordt veroorzaakt doordat patiënten voortdurend moeten letten op wat ze eten, angstig zijn voor een allergische reactie en hierdoor beperkt worden in een gezonde levensstijl, terwijl fruit, noten en peulvruchten juist essentiële onderdelen van een gezond dieet vormen.

Voor volwassenen met een voedselallergie is in Nederland nog geen behandeling beschikbaar, ook niet voor het paraberksyndroom. Omdat het paraberksyndroom erg lijkt op berkenpollenallergie, waarvoor een behandeling beschikbaar is in de vorm van berkenpollen immunotherapie (allergievaccinatie), hebben wij in een literatuurstudie onderzocht of deze behandeling ook effectief is voor het paraberksyndroom (**Hoofdstuk 10**). Hierbij bleek dat er weinig goede studies waren verricht en de resultaten tegenstrijdig waren. Daarom hebben wij onderzocht of een onder de tong toegediende tablet die berkenpollen bevat (sublinguale immunotherapie), een goede behandeling kan zijn voor patiënten met het paraberksyndroom (**Hoofdstuk 11**). Al na een jaar blijkt dat de helft van de patiënten een duidelijke verbetering aangeeft in hun voedselallergie gerelateerde kwaliteit van leven. Tevens rapporteerde 67% van de patiënten een vermindering van de ernst van hun klachten of konden zij een grotere hoeveelheid van het voedingsmiddel verdragen. Gezien deze positieve resultaten zullen de patiënten nog langer worden gevolgd.

Gepersonaliseerde dieetadviezen bij voedselallergie

Omdat er geen standaardbehandeling beschikbaar is voor patiënten met een voedselallergie, worden altijd adviezen gegeven over het behandelen van een allergische reactie met behulp van een noodset en over het te volgen dieet. Omdat patiënten met het paraberksyndroom vaak veel voedingsmiddelen niet kunnen eten, of slechts in een bepaalde (bewerkte) vorm, kunnen gerichte dieetadviezen uitkomst bieden. Het is bekend dat patiënten meestal geen klachten hebben op bewerkte fruit en groenten (zoals appelmoes), maar wel op verse producten. Echter, het is onbekend of dit ook geldt voor noten en soja. In dit proefschrift laten we zien dat patiënten die klachten kregen van rauwe amandel, geen klachten kregen van geroosterde amandel. In deze situatie hoeft geroosterde amandel dus niet vermeden te worden (**Hoofdstuk 4**). Voor hazelnoot, rapporteerde een derde van de patiënten die klachten hadden bij rauwe hazelnoot geen klachten bij geroosterde hazelnoot of hazelnootpasta (**Hoofdstuk 9**).

Voor soja hebben we een soort ranglijst kunnen opstellen van verschillende sojaproducten op basis van de frequentie en ernst van de klachten die deze producten geven. De volgorde was van meest naar minst frequent: sojazuivel, tofu, sojaboon en tempeh, vleesvervanger, sojasaus, brood en koffieleutje. Voor ernst van de klachten was de volgorde redelijk vergelijkbaar: sojazuivel veroorzaakte de meest ernstige klachten, gevolgd door tofu, vleesvervangers, sojaboon en tempeh, sojasaus, brood en koffieleutje. Daarnaast bleek dat bij patiënten die klachten rapporteerden na het gebruik van sojamelk, 53% geen klachten had van andere sojaproducten. De verschillen in frequentie en ernst van klachten tussen sojaproducten, en het feit dat veel patiënten bepaalde sojaproducten wel verdragen, impliceren dat het vermijden van soja in de praktijk individueel en product-specifiek moet worden afgestemd.

Nieuwe kandidaten voor allergenenetikettering

Naast dieetadviezen kan allergenenetikettering van voedingsmiddelen belangrijke handvatten bieden voor mensen met een voedselallergie. In Europa is allergenenetikettering momenteel verplicht voor veertien voedingsmiddelen of groepen voedingsmiddelen, zoals noten, pinda, koemelk, soja, kippenei, schaaldieren, sesam, vis, ontbijtgranen, selderij, mosterd, weekdieren, lupine en sulfaatdioxide/sulfiet.

Voedsel dat op dit moment niet geëtiketteerd wordt, kan frequente en ernstige klachten geven. Wij hebben aangetoond dat pijnboompit 5% van de voedselallergische reacties veroorzaakt en dat in 50,9% van de gevallen sprake is van een ernstige reactie (**Hoofdstuk 2**). Voor zonnebloempit betreft dit respectievelijk 2% en 43%. Op basis van deze resultaten is het te overwegen om pijnboompit in de toekomst te etiketteren en zonnebloempit te plaatsen op de zogenoemde 'watch list' voor mogelijke toekomstige allergenen etikettering.

De belangrijkste conclusies van dit proefschrift zijn

- Bijna 200 verschillende voedingsmiddelen kunnen een voedselallergie veroorzaken
- Zaden en pitten, met name sesamzaad en pijnboompit, veroorzaken het vaakst ernstige reacties, terwijl alleen sesam momenteel verplicht wordt geëtiketteerd
- Het 2S albumine eiwit in cashew is een goede voorspeller voor cashewallergie, terwijl het 2S albumine eiwit in amandel geen betrouwbare voorspeller is voor amandelallergie
- Het perzik allergeen Pru p 7 is zowel in Europa als in Japan de beste voorspeller voor een ernstige perzik allergie
- Continue monitoring van hartslag, bloeddruk, QT-interval, ademhalingsfrequentie en lichaamstemperatuur is veelbelovend en kan in de toekomst een allergische reactie mogelijk eerder voorspellen
- Een thuisprovocatie is veilig mits er aan bepaalde voorwaarden wordt voldaan
- Het paraberksyndroom geeft over het algemeen milde klachten, maar kan in meer dan 10% van de patiënten ernstige klachten veroorzaken.
- Sublinguale immunotherapie met berkenpollen is mogelijk een geschikte therapie voor patiënten met het paraberksyndroom

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LIST OF ABBREVIATIONS

AA	Allergic asthma
AD	Atopic dermatitis
AIT	Allergen immunotherapy
AR	Allergic rhinoconjuctivitis
AUC	Area under the curve
BP	Blood pressure
BMI	Body Mass Index
BPFA	Birch Pollen Food Allergy
CCD	Cross-reactive carbohydrate determinants
CCWS	Checkpoint Cardio Wireless System
CI	Confidence interval
CRD	Component-resolved diagnostics
DBP	Diastolic blood pressure
DBPCFC	Double-blind, placebo controlled food challenge
ED	Eliciting dose
FA	Food allergy
FAQLQ-AF	Food allergy quality of life questionnaire - adult form
FA-QoL	Food allergy-related quality of life
GRP	Gibberellin-regulated protein protein
HR	Heart rate
ICD	Implanted Cardioverter Defibrillator
IgE	Immunoglobulin E
IQR	Inter Quartile Range
kUa/L	Kilo units of antibody per liter
LTP	Lipid transfer protein
MREC	Medical Research Ethics Committee
NA	Not applicable
NL	The Netherlands
NPV	Negative Predictive Value
OAS	Oral Allergy Symptoms
oFASS	Ordinal Food Allergy Severity Score
OFC	Oral food challenge
OR	Odds ratio
PT	Portugal
PTP	Prick to prick
PPV	Positive Predictive Value
PRACTALL	Practical allergy
PR-10	Pathogenesis-related protein family 10

QoL	Quality of life
QT	QT interval
RCT	Randomized controlled trial
RoB2	Revised risk of bias tool
ROC	Receiver operating characteristic
RR	Respiratory rate
SBP	Systolic blood pressure
SCIT	Subcutaneous immunotherapy
SD	Standard deviation
sIgE	Specific immunoglobulin E
SLIT	Sublingual immunotherapy
SpO2	Oxygen saturation
SPT	Skin prick test
SSP	Seed storage proteins
T	Temperature
TLP	Thaumatin-like protein
VAS	Visual analogue scale
UMCU	University Medical Center Utrecht

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LIST OF PUBLICATIONS

This thesis, published

Kallen E.J.J., Welsing P.M.J., Nijenhuis I., et al. Frequency and severity of 192 foods causing food allergy in adults, JACI Global. 2026; 5(2):1-8.

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Emily was born on August 23rd, 1994, in Graft-De Rijp, the Netherlands. At the age of five, she moved to Valkenswaard, where she spent most of her childhood. She attended secondary school at Sint Joris college in Eindhoven, the Netherlands, after which she studied Biomedical Sciences for one year at Maastricht University. At the end of this year, she was invited to participate in the Honours Program in Biomedical Sciences, which provided her with her first real exposure to scientific research and sparked her interest in pursuing research alongside her ambition to become a medical doctor.

In 2014, she started her medical training at Maastricht University, where she particularly enjoyed electives in dermatology. To determine whether gynecology and obstetrics would be a good fit for her, she volunteered at Amana Regional Referral Hospital in Tanzania in the summer of 2019. Although she greatly valued the experience, it confirmed her decision to pursue dermatology. All her elective internships focused on dermatology, resulting in her final year of study being almost exclusively dedicated to this field.

After obtaining her medical degree in 2020, she gained experience as a medical doctor in the dermatology department at Elkerliek hospital in Helmond, the Netherlands, as well as the General Hospital Turnhout and the Da Vinci Clinic in Turnhout, Belgium. In 2021, she started her PhD trajectory in food allergy under the supervision of prof. dr. André Knulst, prof. dr. Ronald van Ree, dr. Thuy-My Le, and dr. Welsing at the University Medical Centre Utrecht (UMCU). In January 2026, she started her dermatology residency program at the same hospital.