A Pan-European Perspective on Food Allergy

Prevalence, Predictors and Patient Profiles

Sarah Lyons

A pan-European perspective on food allergy: prevalence, predictors and patient profiles

Sarah Lyons

A pan-European perspective on food allergy: prevalence, predictors and patient profiles
PhD thesis, Utrecht University, the Netherlands

ISBN	978-90-393-7366-8
Cover design	Sarah Lyons and Frits Erdmann
Lay-out	Sarah Lyons and proefschriftmaken.nl
Printed by	proefschriftmaken.nl

A pan-European perspective on food allergy: prevalence, predictors and patient profiles

Een pan-Europees perspectief op voedselallergie: prevalentie, predictoren en patiëntprofielen
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties in het openbaar te
verdedigen op donderdag 11 maart 2021 des ochtends te 10.30 uur

door

Sarah Anne Lyons
geboren op 30 december 1990 te Cork, Ierland

Promotoren:

Prof. dr. A.C. Knulst

Prof. dr. R. Van Ree

Copromotoren:

Dr. T.T.M. Le

Dr. P.M.J. Welsing

Contents

Chapter 1

General introduction

Typical food allergies are IgE-mediated

Contrary to popular usage,[1] food 'allergy' (FA) is not synonymous to food 'adverse reaction'. The term 'allergy' stems from the Ancient Greek ἄλλος, meaning other or different, and ἔργον, meaning work or activity.[1] At the start of the twentieth century, the term was coined by Austrian paediatrician Von Pirquet to describe a change in reactivity of an individual's immune system upon contact with an antigen.[1] It was not until the late 1960s that scientists discovered the key reagenic antibody involved in allergy: IgE.[1, 2] Although several additional (cell-mediated) immunologic mechanisms for allergic reactions have been described in recent years, FA is first and foremost IgE-mediated.[3, 4] In this thesis, the term FA refers to symptoms initiated by IgE production in response to exposure to a food at a dose tolerated by non-allergic individuals.[3, 4]

Establishing the prevalence of FA is a challenging task

Most studies report the prevalence of *self-reported FA*.[5-8] However, fashionable use of the term allergy to cover any adverse food reaction,[1] carries the unavoidable risk of overestimation of prevalence of FA based on self-reported reactions.[7, 9] Previously published prevalence estimates of self-reported FA range from 3% to as high as 35%.[5, 7]

Prevalence of FA is ideally determined based on oral food challenge, preferably double-blind placebo-controlled food challenge (DBPCFC).[9] DBPCFC is considered the 'gold standard' test. During DBPCFC, the offending food is administered orally in gradually increasing doses, and reactivity is compared to placebo.[9-12] Based on systematic review and meta-analysis, the pooled prevalence *of challenge-confirmed FA* in Europe is estimated to be around 1% (range 0.3-5.7%).[7] However, the accuracy of prevalence estimates based on food challenge is also subject to scrutiny. Food challenge can still lead to overestimation, because diagnosis largely depends on subjective reporting of symptoms, or because flare-up of other spontaneous conditions, such as chronic urticaria or allergic asthma, due to medication withdrawal, may be misinterpreted as a food-induced reaction.[13] Alternatively, underestimation of prevalence is possible, because of exclusion and stopping criteria, performance in a setting unrepresentative of real-life conditions, or rejection of the time-consuming and burdensome test in favour of avoidance by the patient.[13]

As a prerequisite for FA*, food sensitisation* (FS) can also help narrow down the food allergic population, although it does not invariably lead to clinical symptoms of allergy in itself. FS entails the presence of IgE antibodies against the culprit food. The combined presence of symptoms and matching IgE sensitisation to the culprit food is termed *probable FA* in this thesis, in keeping with previously used terminology by the European Academy of Allergy and Clinical Immunology (EAACI) task force.[9] Data on the prevalence of probable FA in Europe are scarce,[7] but it is a key prevalence

estimate, considering that probable FA is often the best attainable endpoint in daily practice. An overall estimate of around 2% has been reported for German adults,[14] and estimates ranging from 0.1% to 13% in school-age children from Germany,[15] France[16] and Turkey[17].

Besides depending on the chosen outcome definition, prevalence of FA also seems to be related to age, geographical location, and time period.[5-7] FA is suggested to be more common in children than in adults, to occur more frequently in Northern and Western than in Southern Europe, and to be increasing in prevalence over time.[7] These variations suggest environmental influences on the development of FA.[18] However, the extent of variation in the prevalence of FA remains unclear, and the role of environmental factors is difficult to establish, because of considerable methodological heterogeneity among studies conducted in different populations and settings (e.g. different sampling methods and evaluated foods). This is where the EuroPrevall project comes in.

The EuroPrevall project yielded data collected according to a standardised approach across Europe

As part of the EU-funded EuroPrevall research project, three unique patient cohorts were prospectively established from 2005 onwards, and evaluated in parallel all across Europe, using predetermined standardised protocols.[19-22] For the birth cohort, newborns were recruited from nine countries and followed until the age of 2.5 years.[20] For the cross-sectional community surveys, school-age children (7-10 years) and adults (18-54 years) from the general population of eight countries were approached.[21] To complement these population-based studies, a cross-sectional study was also conducted in an outpatient population, which included patients of any age referred to allergy clinics with a suspected FA in 12 countries.[22] The different countries were selected to represent major cultural and climatic regions of Europe. The community surveys focused particularly on 24 foods commonly implicated in FA or frequently consumed in participating countries (the so-called EuroPrevall *priority foods*): cow's milk, hen's egg, fish, shrimp, hazelnut, walnut, peach, apple, kiwi, melon, banana, tomato, celery, carrot, peanut, soy, lentils, wheat, buckwheat, corn, sesame seed, mustard seed, sunflower seed, and poppy seed.

As the name of the project communicates, one of the main aims of EuroPrevall was to establish the true variation in the prevalence of FAs across Europe.[21] This primary goal was accomplished in ***Chapter 2*** of this thesis, in which prevalence of self-reported FA, FS, and probable FA were determined in school-age children, and in ***Chapter 3***, in which prevalence of probable FA was established in adults. Available data on challenge-confirmed FA are also presented in these chapters. For adults, EuroPrevall prevalence estimates of self-reported FA and FS were previously published to range from respectively 1 to 19% and 7 to 24%.[23]

The second objective of the EuroPrevall community surveys was to generate knowledge on the relationship between suspected risk factors or environmental determinants and (development of) FAs.[20, 21] The EAACI FA guidelines state that the prevalence of secondary FA caused by cross-reactions of food allergens with inhalant allergens appears to be increasing. [9] In ***Chapter 2 and 3***, we discuss how sensitisation to pollen may affect observed patterns of FS and FA. In ***Chapter 4***, we describe the associations between mainly early-life environmental exposures (e.g. sibship size, day care attendance, pets, growing up in a farm environment, infant diet) and FS in both school-age children and adults.

To date, no *in vivo* or *in vitro* diagnostic tests correlate fully with clinical FA

As indicated before, the gold standard test for diagnosing FA is oral food challenge.[9] However, improvement of the predictive value of other steps in the diagnostic work-up of FA is strongly desired, to reduce the need for resource-intensive and burdensome challenge tests.

Patient history is considered the first step in and the main tool for diagnosis of FA.[9, 24] According to guidelines, timing and reproducibility of the reaction, symptoms, and co-existing allergic diseases should be addressed.[9, 25, 26] However, besides acknowledging the importance of patient history for diagnosing FA, EAACI guidelines also stress the need for studies evaluating prediction of FA using standardised allergy-focused history questionnaires, because current evidence is based on expert opinion.[9, 25] The EuroPrevall data were used to tackle this knowledge gap in ***Chapter 5***, where we ascertained which reaction characteristics, allergic comorbidities and demographic factors contribute to optimal prediction of probable FA in adults and school-age children reporting adverse reactions to foods.

On top of patient history, routine diagnostic tests for FA in current daily practice include extract-based skin prick testing (SPT) *in vivo* and extract-based serum IgE testing *in vitro*. Both aim to establish or rule out FS. However, extract-based testing can fall short because commercially available food extracts do not accurately represent the allergenic composition of the fresh food.[27] For example, enzymatic oxidative processes, pH, and defatting procedures can respectively reduce concentration of pathogenesis related protein family 10 (PR-10) proteins, lipid transfer proteins (LTP), and lipophilic proteins like oleosins in food extracts compared to the native food.[28] Prick-to-prick (PTP) testing with fresh foods is not subject to these shortcomings of extract-based testing and, although poorly standardised, is sometimes applied to increase sensitivity of *in vivo* testing.[29] Regarding serology testing, attempts have been made to improve sensitivity of extract-based tests, for example by spiking hazelnut extract with hazelnut PR-10 protein Cor a 1.[29, 30] Nonetheless, detection of clinically relevant FS remains suboptimal. Furthermore, it is important to realise that detection of clinically irrelevant IgE sensitisation is also a

cause for concern. Particularly noteworthy is that IgE against cross-reactive carbohydrate determinants in food is detected in up to 70% of pollen allergic subjects, but is not associated with food allergic symptoms.[31-34]

With the aim of improving predictive value of the FA diagnostic work-up, serum IgE testing using whole food extracts has been complemented with component-resolved diagnostics (CRD) in recent years. CRD involves measurement of IgE antibodies against individual allergenic molecules.[9] Besides the fact that CRD can improve sensitivity with respect to allergen components that are underrepresented in extract, CRD can help discriminate between primary and cross-reactive sensitisation, and potentially assist in prediction of (clinical phenotype of) FA.[29] One of the most widely recognised examples is that sensitisation to plant source food PR-10 proteins almost exclusively occurs as a result of cross-reactivity with major birch pollen Bet v 1, and is generally associated with tolerance or mild (typically oral allergy) symptoms.[35-37] Contrastingly, sensitisation to plant source food storage proteins is thought to be associated with allergy, possibly severe allergy. Most evidence is available for peanut 2S albumin Ara h 2, which has been demonstrated to accurately distinguish peanut allergy from tolerance to peanut,[38, 39] and is linked to a severe phenotype in several studies.[40] Hazelnut 11S globulin Cor a 9 and 2S albumin Cor a 14, both storage proteins, are also thought to be markers of hazelnut allergy,[41-43] and of a severe hazelnut allergy phenotype.[44-46] However, data on the diagnostic accuracy of hazelnut CRD for predicting hazelnut allergy and severity of hazelnut allergy in an unselected adult population are still lacking. For this reason, we investigated this topic in all adults who consecutively underwent DBPCFC with hazelnut between 2012 and 2019 at the University Medical Centre of Utrecht in the Netherlands, as described in ***Chapter 6***.

Perhaps the key to accurate prediction of (severity of) allergy lies in combining the most relevant information from patient history, extract-based testing, and CRD. A recent study evaluated data from clinical background in combination with extract-based testing and CRD results for predicting severity of hazelnut allergy in the mixed paediatric and adult EuroPrevall outpatient population. The resulting model combining atopic dermatitis (ever), pollen allergy, IgE to walnut extract, and IgE to hazelnut Cor a 14, more accurately estimated the risk of severe hazelnut allergy than clinical background, extract-based testing, or CRD alone.[45] In ***Chapter 7 and 8***, we performed similar analyses for prediction of severity of walnut and peanut allergy respectively, using data from the EuroPrevall outpatient study.

Dietary approaches to highly prevalent pollen-related FA lack uniformity

Once (severity of) FA is established, appropriate dietary avoidance is considered the key intervention in the management of FA.[9] Clinical guidelines state that dietary restrictions should eliminate the culprit food allergen(s) to the level of the eliciting

dose and should be tailored to the individual's allergic and nutritional needs.[9] As will become increasingly clear throughout this thesis, FA resulting from cross-reactivity with pollen is one of the most common types of plant FA in older children and adults across Europe, especially birch pollen-related FA in Northern and Central Europe.[35, 47] Since prevalence of pollen allergy is increasing, a continuing rise in the prevalence of pollen-related FA is also expected.[35, 47] Pollen-related FA usually presents with mild symptoms,[35-37, 47-49] and the proteins mostly responsible for pollen-related FAs in Europe include PR-10 proteins and profilins, which are heat- and digestion-labile.[33, 34] As such, more lenient and explorative dietary advice may be suited to this particular condition. Current guidelines give no specific suggestions on avoidance of traces, cross-reacting foods or foods within the same family in the case of pollen-related FA. Of course dietary avoidance advice should be tailored to the patient, but lack of uniform guidelines cause avoidance recommendations in similar patients to differ per physician.[50] Furthermore, the clinical efficacy of other dietary interventions, such as oral immunotherapy (OIT) with food, heat processing, and consumption of low allergenic cultivars on pollen-related FA, is unknown. To provide an overview of available evidence as a base for creating a more standardised therapeutic approach to pollen-related FA, we dedicated ***Chapter 9*** to a systematic review on dietary interventions for this condition.

A lot remains to be discovered on the topic of FA across Europe

The chapters of this thesis will reveal the true geographical differences in prevalence of FS and FA in children and adults across Europe; provide insight into the associations between (early-life) environmental exposures and FS; describe the aspects of patient history, extract-based testing and CRD contributing to prediction of (severity of) FA; and finally discuss dietary interventions for a particularly prevalent type of FA in (North-Western) Europe, (birch-)pollen related FA. The implications of our findings and considerations for future research will be explored in ***Chapter 10***.

References

1. Igea JM. The history of the idea of allergy. Allergy. 2013;68(8):966-73.
2. Immunoglobulin E, a new class of human immunoglobulin. Bull World Health Organ. 1968;38(1):151-2.
3. Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy. 2001;56(9):813-24.
4. Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol. 2004;113(5):832-6.
5. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. J Allergy Clin Immunol. 2007;120(3):638-46.
6. Zuidmeer L, Goldhahn K, Rona RJ, Gislason D, Madsen C, Summers C, et al. The prevalence of plant food allergies: a systematic review. J Allergy Clin Immunol. 2008;121(5):1210-8 e4.
7. Nwaru BI, Hickstein L, Panesar SS, Muraro A, Werfel T, Cardona V, et al. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. Allergy. 2014;69(1):62-75.
8. Nwaru BI, Hickstein L, Panesar SS, Roberts G, Muraro A, Sheikh A, et al. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. Allergy. 2014;69(8):992-1007.
9. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy. 2014;69(8):1008-25.
10. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. Standardization of food challenges in patients with immediate reactions to foods--position paper from the European Academy of Allergology and Clinical Immunology. Allergy. 2004;59(7):690-7.
11. Taylor SL, Hefle SL, Bindslev-Jensen C, Atkins FM, Andre C, Bruijnzeel-Koomen C, et al. A consensus protocol for the determination of the threshold doses for allergenic foods: how much is too much? Clin Exp Allergy. 2004;34(5):689-95.
12. Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, Sicherer S, Teuber SS, Burks AW, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report. J Allergy Clin Immunol. 2012;130(6):1260-74.
13. Asero R, Fernandez-Rivas M, Knulst AC, Bruijnzeel-Koomen CA. Double-blind, placebo-controlled food challenge in adults in everyday clinical practice: a reappraisal of their limitations and real indications. Curr Opin Allergy Clin Immunol. 2009;9(4):379-85.
14. Zuberbier T, Edenharter G, Worm M, Ehlers I, Reimann S, Hantke T, et al. Prevalence of adverse reactions to food in Germany - a population study. Allergy. 2004;59(3):338-45.
15. Roehr CC, Edenharter G, Reimann S, Ehlers I, Worm M, Zuberbier T, et al. Food allergy and non-allergic food hypersensitivity in children and adolescents. Clin Exp Allergy. 2004;34(10):1534-41.
16. Penard-Morand C, Raherison C, Kopferschmitt C, Caillaud D, Lavaud F, Charpin D, et al. Prevalence of food allergy and its relationship to asthma and allergic rhinitis in schoolchildren. Allergy. 2005;60(9):1165-71.
17. Orhan F, Karakas T, Cakir M, Aksoy A, Baki A, Gedik Y. Prevalence of immunoglobulin E-mediated food allergy in 6-9-year-old urban schoolchildren in the eastern Black Sea region of Turkey. Clin Exp Allergy. 2009;39(7):1027-35.
18. Papadopoulos NG, Agache I, Bavbek S, Bilo BM, Braido F, Cardona V, et al. Research needs in allergy: an EAACI position paper, in collaboration with EFA. Clin Transl Allergy. 2012;2(1):21.
19. Mills EN, Mackie AR, Burney P, Beyer K, Frewer L, Madsen C, et al. The prevalence, cost and basis of food allergy across Europe. Allergy. 2007;62(7):717-22.
20. Keil T, McBride D, Grimshaw K, Niggemann B, Xepapadaki P, Zannikos K, et al. The multinational birth cohort of EuroPrevall: background, aims and methods. Allergy. 2010;65(4):482-90.

21. Kummeling I, Mills EN, Clausen M, Dubakiene R, Perez CF, Fernandez-Rivas M, et al. The EuroPrevall surveys on the prevalence of food allergies in children and adults: background and study methodology. Allergy. 2009;64(10):1493-7.
22. Fernandez-Rivas M, Barreales L, Mackie AR, Fritsche P, Vazquez-Cortes S, Jedrzejczak-Czechowicz M, et al. The EuroPrevall outpatient clinic study on food allergy: background and methodology. Allergy. 2015;70(5):576-84.
23. Burney PG, Potts J, Kummeling I, Mills EN, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitization in European adults. Allergy. 2014;69(3):365-71.
24. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol. 2018;141(1):41-58.
25. Skypala IJ, Venter C, Meyer R, deJong NW, Fox AT, Groetch M, et al. The development of a standardised diet history tool to support the diagnosis of food allergy. Clin Transl Allergy. 2015;5:7.
26. Sampson HA. Food allergy--accurately identifying clinical reactivity. Allergy. 2005;60 Suppl 79:19-24.
27. Lidholm J, Ballmer-Weber BK, Mari A, Vieths S. Component-resolved diagnostics in food allergy. Curr Opin Allergy Clin Immunol. 2006;6(3):234-40.
28. Amlot PL, Kemeny DM, Zachary C, Parkes P, Lessof MH. Oral allergy syndrome (OAS): symptoms of IgE-mediated hypersensitivity to foods. Clin Allergy. 1987;17(1):33-42.
29. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, et al. EAACI Molecular Allergology User's Guide. Pediatr Allergy Immunol. 2016;27 Suppl 23:1-250.
30. Sicherer SH, Dhillon G, Laughery KA, Hamilton RG, Wood RA. Caution: the Phadia hazelnut ImmunoCAP (f17) has been supplemented with recombinant Cor a 1 and now detects Bet v 1-specific IgE, which leads to elevated values for persons with birch pollen allergy. J Allergy Clin Immunol. 2008;122(2):413-4, 4 e2.
31. Mari A. IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. Int Arch Allergy Immunol. 2002;129(4):286-95.
32. Altmann F. Coping with cross-reactive carbohydrate determinants in allergy diagnosis. Allergo J Int. 2016;25(4):98-105.
33. Hauser M, Roulias A, Ferreira F, Egger M. Panallergens and their impact on the allergic patient. Allergy Asthma Clin Immunol. 2010;6(1):1.
34. McKenna OE, Asam C, Araujo GR, Roulias A, Goulart LR, Ferreira F. How relevant is panallergen sensitization in the development of allergies? Pediatr Allergy Immunol. 2016;27(6):560-8.
35. Werfel T, Asero R, Ballmer-Weber BK, Beyer K, Enrique E, Knulst AC, et al. Position paper of the EAACI: food allergy due to immunological cross-reactions with common inhalant allergens. Allergy. 2015;70(9):1079-90.
36. Blankestijn MA, Knulst AC, Knol EF, Le TM, Rockmann H, Otten HG, et al. Sensitization to PR-10 proteins is indicative of distinctive sensitization patterns in adults with a suspected food allergy. Clin Transl Allergy. 2017;7:42.
37. Geroldinger-Simic M, Zelniker T, Aberer W, Ebner C, Egger C, Greiderer A, et al. Birch pollen-related food allergy: clinical aspects and the role of allergen-specific IgE and IgG4 antibodies. J Allergy Clin Immunol. 2011;127(3):616-22 e1.
38. Nilsson C, Berthold M, Mascialino B, Orme ME, Sjolander S, Hamilton RG. Accuracy of component-resolved diagnostics in peanut allergy: Systematic literature review and meta-analysis. Pediatr Allergy Immunol. 2020;31(3):303-14.
39. Hemmings O, Du Toit G, Radulovic S, Lack G, Santos AF. Ara h 2 is the dominant peanut allergen despite similarities with Ara h 6. J Allergy Clin Immunol. 2020.
40. Buyuktiryaki B, Santos AF. Food allergy severity predictions based on cellular in vitro tests. Expert Rev Mol Diagn. 2020; 146(3): 621-630.e5.
41. Beyer K, Grabenhenrich L, Hartl M, Beder A, Kalb B, Ziegert M, et al. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. Allergy. 2015;70(1):90-8.

42. Buyuktiryaki B, Cavkaytar O, Sahiner UM, Yilmaz EA, Yavuz ST, Soyer O, et al. Cor a 14, Hazelnut-Specific IgE, and SPT as a Reliable Tool in Hazelnut Allergy Diagnosis in Eastern Mediterranean Children. J Allergy Clin Immunol Pract. 2016;4(2):265-72 e3.
43. Brandstrom J, Nopp A, Johansson SG, Lilja G, Sundqvist AC, Borres MP, et al. Basophil allergen threshold sensitivity and component-resolved diagnostics improve hazelnut allergy diagnosis. Clin Exp Allergy. 2015;45(9):1412-8.
44. Masthoff LJ, Mattsson L, Zuidmeer-Jongejan L, Lidholm J, Andersson K, Akkerdaas JH, et al. Sensitization to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. J Allergy Clin Immunol. 2013;132(2):393-9.
45. Datema MR, van Ree R, Asero R, Barreales L, Belohlavkova S, de Blay F, et al. Component-resolved diagnosis and beyond: Multivariable regression models to predict severity of hazelnut allergy. Allergy. 2018;73(3):549-59.
46. Faber MA, De Graag M, Van Der Heijden C, Sabato V, Hagendorens MM, Bridts CH, et al. Cor a 14: missing link in the molecular diagnosis of hazelnut allergy? Int Arch Allergy Immunol. 2014;164(3):200-6.
47. Worm M, Jappe U, Kleine-Tebbe J, Schafer C, Reese I, Saloga J, et al. Food allergies resulting from immunological cross-reactivity with inhalant allergens: Guidelines from the German Society for Allergology and Clinical Immunology (DGAKI), the German Dermatology Society (DDG), the Association of German Allergologists (AeDA) and the Society for Pediatric Allergology and Environmental Medicine (GPA). Allergo J Int. 2014;23(1):1-16.
48. Hofmann A, Burks AW. Pollen food syndrome: update on the allergens. Curr Allergy Asthma Rep. 2008;8(5):413-7.
49. Santos A, Van Ree R. Profilins: mimickers of allergy or relevant allergens? Int Arch Allergy Immunol. 2011;155(3):191-204.
50. Ma S, Sicherer SH, Nowak-Wegrzyn A. A survey on the management of pollen-food allergy syndrome in allergy practices. J Allergy Clin Immunol. 2003;112(4):784-8.

PART

Food allergy in the general population

prevalence patterns and potential risk factors

Chapter 2

Prevalence of food sensitisation and food allergy in children across Europe

J Allergy Clin Immunol Pract. 2020; 8(8): 2736-2746

Sarah A. Lyons,* Michael Clausen,* André C. Knulst, Barbara K. Ballmer-Weber, Montserrat Fernández-Rivas, Laura Barreales, Christian Bieli, Ruta Dubakiene, Cristina Fernández-Pérez, Monika Jedrzejczak-Czechowicz, Marek L. Kowalski, Tanya Kralimarkova, Ischa Kummeling, Tihomir B. Mustakov, Nikolaos G. Papadopoulos, Todor A. Popov, Paraskevi Xepapadaki, Paco M.J. Welsing, James Potts, E.N. Clare Mills, Ronald van Ree, Peter G.J. Burney,§ Thuy-My Le§

* § *Contributed equally*

Abstract

Background
For adults, prevalence estimates of food sensitisation (FS) and food allergy (FA) have been obtained in a standardised manner across Europe. For children, such estimates are lacking.

Objective
To determine the prevalence of self-reported FA, FS, probable FA (symptoms plus IgE sensitisation), and challenge-confirmed FA in European school-age children**.**

Methods
Data on self-reported FA were collected using a screening questionnaire sent to a random sample of the general population of 7- to 10-year-old children in 8 European centres in phase I of the EuroPrevall study. Data on FS and probable FA were obtained in phase II, comprising an extensive questionnaire on reactions to 24 commonly implicated foods, and serology testing. Food challenge was performed in phase III.

Results
Prevalence (95%-CI) of self-reported FA ranged from 6.5% (5.4-7.6) in Athens to 24.6% (22.8-26.5) in Lodz; prevalence of FS ranged from 11.0% (9.7-12.3) in Reykjavik to 28.7% (26.9-30.6) in Zurich; and prevalence of probable FA ranged from 1.9% (0.8-3.5) in Reykjavik to 5.6% (3.6-8.1) in Lodz. In all centres, the majority of food-sensitised subjects had primary (non-cross-reactive) FS. However, FS due to birch pollen related cross-reactivity was also common in Central-Northern Europe. Probable FA to cow's milk and hen's egg occurred frequently throughout Europe. Probable FA to fish and shrimp was observed relatively most often in the Mediterranean and Reykjavik. Peach, kiwi and peanut were prominent sources of plant FA in most countries, along with notably hazelnut, apple, carrot and celery in Central-Northern Europe, and lentils and walnut in the Mediterranean.

Conclusions
There are large geograhical differences in prevalence of FS and FA in school-age children across Europe. Both primary and cross-reactive FS and FA occur frequently.

Introduction

Prevalence of food allergy (FA) in children from European countries has been evaluated in several studies, using various study designs and outcome definitions. Studies published between 2000 and 2012 reveal estimates ranging from 5.7% to 41.8% for lifetime prevalence of *self-reported FA*, and from 1.6% to 24.4% for point prevalence of *self-reported FA* in 6- to 10-year old European children.[1] Point prevalence of *food sensitisation* (FS), which entails the presence of IgE antibodies against specific foods, and is a prerequisite for IgE-mediated FA, varies between 4.1% and 52.0% in the same age group.[1] The combination of typical clinical symptoms and IgE sensitisation to the same food, which is required for FA diagnosis, is consensually termed *probable FA* by the European Academy of Allergy and Clinical Immunology.[2] The point prevalence of *probable FA* was found to be 4.6% in children of any age in a German study.[1, 3] *Confirmed FA*, based on open or double-blind placebo-controlled food challenge (DBPCFC), is reported to occur in 0.4% to 4.2% of 6- to 10-year-old children in Europe.[1]

It is clear that reported prevalence estimates vary considerably, even between studies using the same definition of FA in similar age groups. A likely explanation is that there are geographical differences in prevalence and causative foods across Europe. However, the extent of these differences remains unclear due to methodological heterogeneity among studies conducted in different countries (e.g. sampling methods and evaluated foods).

In adults, data from the well-standardised pan-European EuroPrevall project have permitted valid comparisons of FA prevalence estimates in multiple European countries. Analyses of these data have revealed the true geographical variation in the prevalence of FA in the European general adult population, and the foods involved.[4, 5] Prevalence of self-reported FA in adults was found to range from around 1.0 % to 18.9% for commonly implicated foods; prevalence of FS from 6.6% to 23.6%; and prevalence of probable FA from 0.3% to 5.6%, with plant source foods dominating as causative foods.

In the current study, data collected during the EuroPrevall project from the general population of 7- to 10-year old European children were evaluated, to provide prevalence estimates of self-reported FA, FS, probable FA and confirmed FA, and corresponding symptoms and causative foods. A distinction was made between animal and plant source foods, because pollen-related cross-reactivity may play a role in the latter.

Methods

Study design

The 3 phases of the multicentre cross-sectional EuroPrevall study were described in detail previously.[6, 7] Briefly, in phase I, a screening questionnaire was distributed to randomly sampled 7- to 10-year-old children from the general population of Zurich (Switzerland), Madrid (Spain), Athens (Greece), Sofia (Bulgaria), Lodz (Poland), Vilnius (Lithuania), Reykjavik (Iceland) and Utrecht (the Netherlands). Twenty-four foods commonly implicated in FA, or often consumed in participating countries, were deemed so-called *priority foods:* cow's milk, hen's egg, fish, shrimp, peanut, hazelnut, walnut, peach, apple, kiwi, melon, banana, tomato, celery, carrot, corn, lentils, soy, wheat, buckwheat, sesame seed, mustard seed, sunflower seed, and poppy seed. In phase II, responders reporting symptoms to 1 or more of these priority foods (cases) and a random sample of responders who did not report symptoms to any of the priority foods (controls), answered a more extensive questionnaire and underwent blood sampling to test for IgE to priority foods and common inhalant allergens. In phase III, DBPCFC was offered to subjects with self-reported symptoms and matching IgE to 1 of 9 priority foods selected for challenge testing (cow's milk, hen's egg, fish, shrimp, peanut, hazelnut, apple, peach, and celery).

All participating centres obtained local ethical approval, and all participants provided informed consent. All phase I, II and III evaluations were completed between 2007 and 2009, with a median time interval of 5 months between phase I and II, and 7 months between phase II and III.

Outcome definitions

The prevalence of the following FA definitions was explored:

I. *Self-reported FA:* symptoms ever reported to any food, and to any priority food.

II. *Food sensitisation:* positive IgE serology (IgE ≥0.35 kU_A/L) for at least 1 of the 24 priority foods. FS was considered primary FS if positive IgE serology was not due to cross-reactivity with pollen (Figure S1). Prevalence of primary FS was also established.

III. *Probable FA:* self-reported FA in combination with matching positive IgE serology (IgE ≥0.35 kU_A/L) for at least 1 of the 24 priority foods.

IV. *Confirmed FA*: DBPCFC-confirmed FA to at least 1 of the 9 foods selected for challenge testing.

Further information on data collection is given in the 'Supplemental methods on data collection'.

Statistical analysis

Based on data from phase I, the prevalence of self-reported FA was calculated as the percentage of responders reporting symptoms to any food, and to at least 1 priority food. Data from phase II were used to estimate the prevalence of FS and probable FA. The percentages of subjects with these outcomes were weighted back according to the sampling scheme in each centre (see supplemental 'Weighting procedure for population prevalence estimation of probable FA'; see Figure S2). Only subjects with available food serology were included. Subjects with discrepancies in the clinical questionnaires of phase I and II were excluded for calculation of probable FA (because of uncertainties regarding symptomatology), but were included in the study population for calculation of FS. The Bulgarian site Sofia was excluded from analysis beyond phase I, because very few subjects participated in phase II (only 16 cases and 9 controls) to result in valid prevalence estimations.

Further exploration included examination of cross-reactivity in subjects sensitised to plant source foods, where a distinction was made between subjects with only primary sensitisation, likely pathogenesis-related protein family 10 (PR-10) cross-reactivity, likely profilin/cross-reactive carbohydrate determinant (CCD) cross-reactivity, or a combination of such sensitisation patterns (Figure S1).

Regarding confirmed FA, phase III data yielded the number and percentage of subjects challenged with each of the 9 selected foods and the frequency of positive challenge test results. No prevalence estimates could be obtained because of the low number of challenges.

Analyses were conducted with SPSS version 25 (IBM Corporation, Armonk, NY) and R version 3.4.1 (R Core Team, Vienna, Austria).

Results

Phase I - Self-reported FA

As shown in Figure 1, 16.935 subjects (59.2%) responded to the phase I screening questionnaire. Participating subjects had a mean age of 8.9 years, and 50.1% were males. The prevalence of self-reported FA varied considerably between centres, ranging from 13.1% to 47.5% for any food and from 6.5% to 24.6% for priority foods (Figure 2). Prevalence was lowest in Athens, and notably high in Vilnius and Lodz. The priority foods most commonly reported for self-reported FA in the overall population were cow's milk (20.3%), hen's egg (9.9%), tomato (5.2%), fish (3.6%), kiwi (2.9%), apple (2.1%), peanut (1.9%), wheat (1.7%), carrot (1.1%), and banana (1.1%). Self-reported FA to nonpriority foods, of which chocolate (13.0%), strawberry (5.8%), and orange (4.4%) were most often specified as causative foods, was particularly common in Vilnius and Lodz.

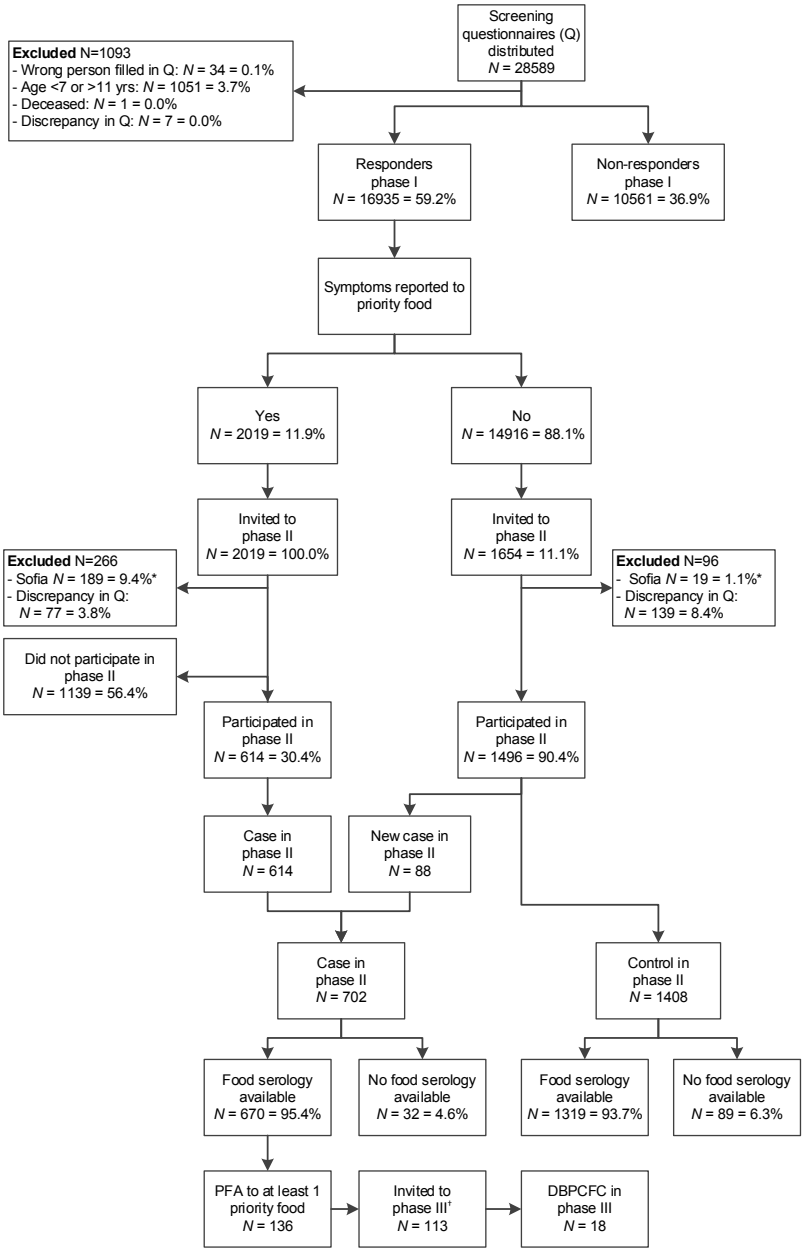

Figure 1. Flowchart

Overall participation in phase I, II and III of the EuroPrevall population-based study in school-age children. Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but symptoms to only nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I. *Sofia was excluded from calculation of probable FA prevalence because of lack of cases participating in phase I. † Probable FA to cow's milk, hen's egg, fish, shrimp, peanut, hazelnut, apple, peach, or celery. *PFA, probable food allergy; DBPCFC, double-blind placebo-controlled food challenge.*

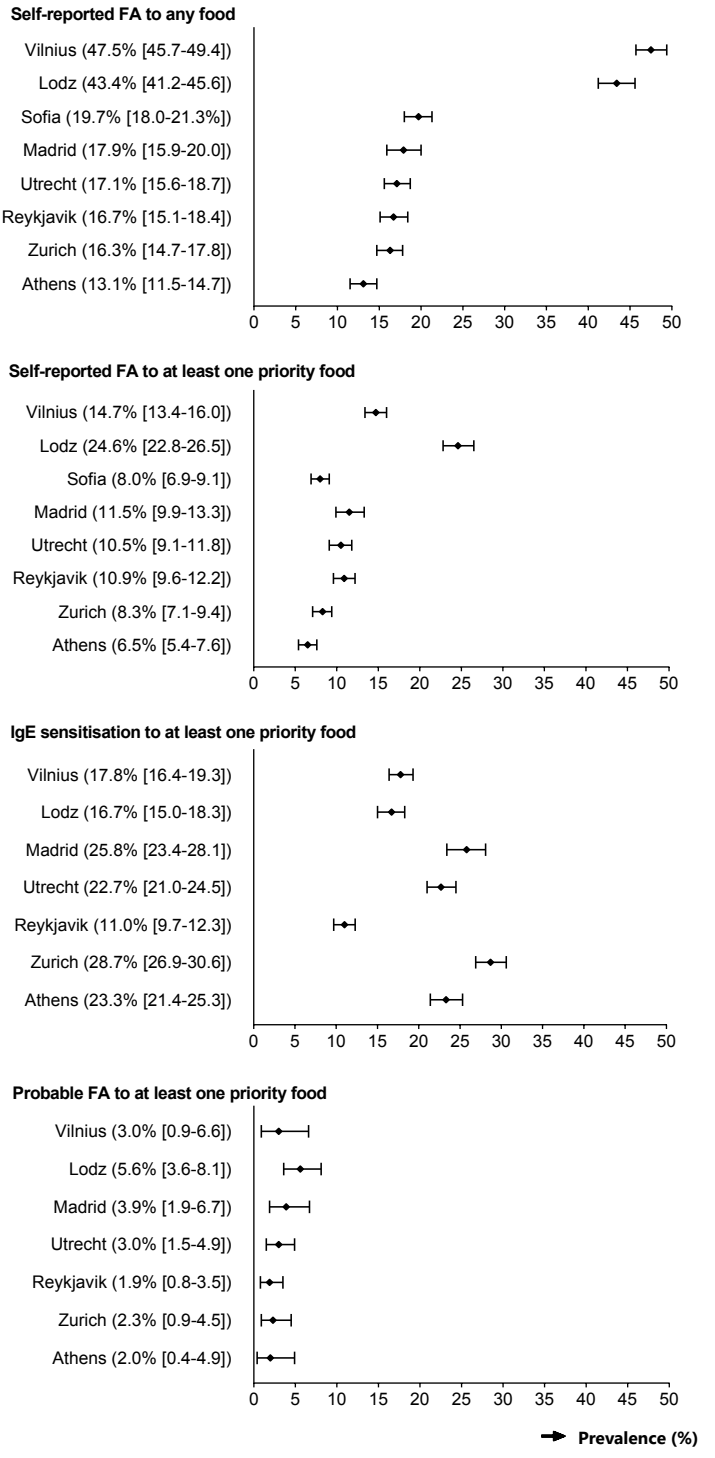

Figure 2. Prevalence of probable FA compared with prevalence of self-reported FA and prevalence of FS

In both subjects with self-reported FA to any food and self-reported FA to priority foods, skin symptoms (61.6% and 70.2%, respectively) and gastrointestinal symptoms (39.5% and 37.3%, respectively) were reported most frequently (Table 1). Notably, oral allergy symptoms, which are generally the first symptoms subjects with an IgE-mediated FA experience,[8, 9] were only rarely reported in relation to self-reported FA in North-Eastern Europe (Vilnius, Lodz), that is, 5.8% to 6.5% for any food compared with 16.3% on average over all centres, and 8.1% to 9.2% for priority foods compared with 23.4% on average.

Table 1. Reported symptoms for self-reported and probable FA

	Self-reported FA to any food (N=4265)	**Self-reported FA to priority food (N=2019)**	**Probable FA to priority food (N=136)**
Age in years, *mean (±SD)*	8.89 (±1.01)	8.85 (±1.01)	9.02 (±0.99)
Male sex	2116 (49.7)	1014 (50.3)	68 (50.0)
Oral allergy symptoms	631 (16.3)	438 (23.4)	75 (56.0)
Isolated oral allergy symptoms	122 (3.2)	89 (4.7)	7 (5.2)
Skin symptoms	2456 (61.6)	1344 (70.2)	108 (80.6)
Rhinoconjunctivitis	959 (24.7)	534 (28.6)	55 (42.0)
Gastrointestinal symptoms	1567 (39.5)	711 (37.3)	38 (29.5)
Difficulty swallowing	200 (5.2)	110 (5.9)	25 (19.2)
Respiratory symptoms	290 (7.5)	186 (10.0)	27 (20.8)
Cardiovascular symptoms	111 (2.9)	48 (2.6)	6 (4.6)
Other symptoms	1224 (31.4)	624 (33.4)	48 (37.2)
Lifetime frequency of reactions			
1x	1x	986 (24.4)	276 (14.1)
2-4x	2-4x	1315 (32.6)	540 (27.6)
>4x	>4x	1738 (43.0)	1137 (58.2)
Previous doctor-diagnosis of FA	1671 (40.2)	1128 (56.9)	94 (70.1)

Values are N (%) unless otherwise indicated. Oral allergy symptoms: itching/tingling/swelling of the mouth/lips/throat. Skin symptoms: rash/nettle sting/itchy skin. Rhinoconjunctivitis: runny/stuffy nose or red/sore/running eyes. Gastrointestinal symptoms: diarrhoea/vomiting. Respiratory symptoms: breathlessness. Cardiovascular symptoms: fainting/dizziness. Other symptoms: stiffness in joints or headaches or other symptoms.

Phase II – Food sensitisation

Prevalence of FS was estimated through evaluation of 2196 subjects with available food serology participating in phase II. Figure 2 shows that prevalence estimates of FS ranged from 11.0% in Reykjavik to 28.7% in Zurich. Although prevalence estimates for each specific food varied substantially between centres, there was considerable overlap in the most common causative foods, as seen in Figure 3. The foods most frequently causing FS in the different centres included animal source foods cow's milk and hen's egg and plant-source foods banana, wheat, hazelnut, apple, peach, kiwi, tomato, celery, carrot, sesame seed, and peanut. Prevalence estimates of FS for all priority foods are available in Table S1. Fish was one of the least common sensitisers in all countries.

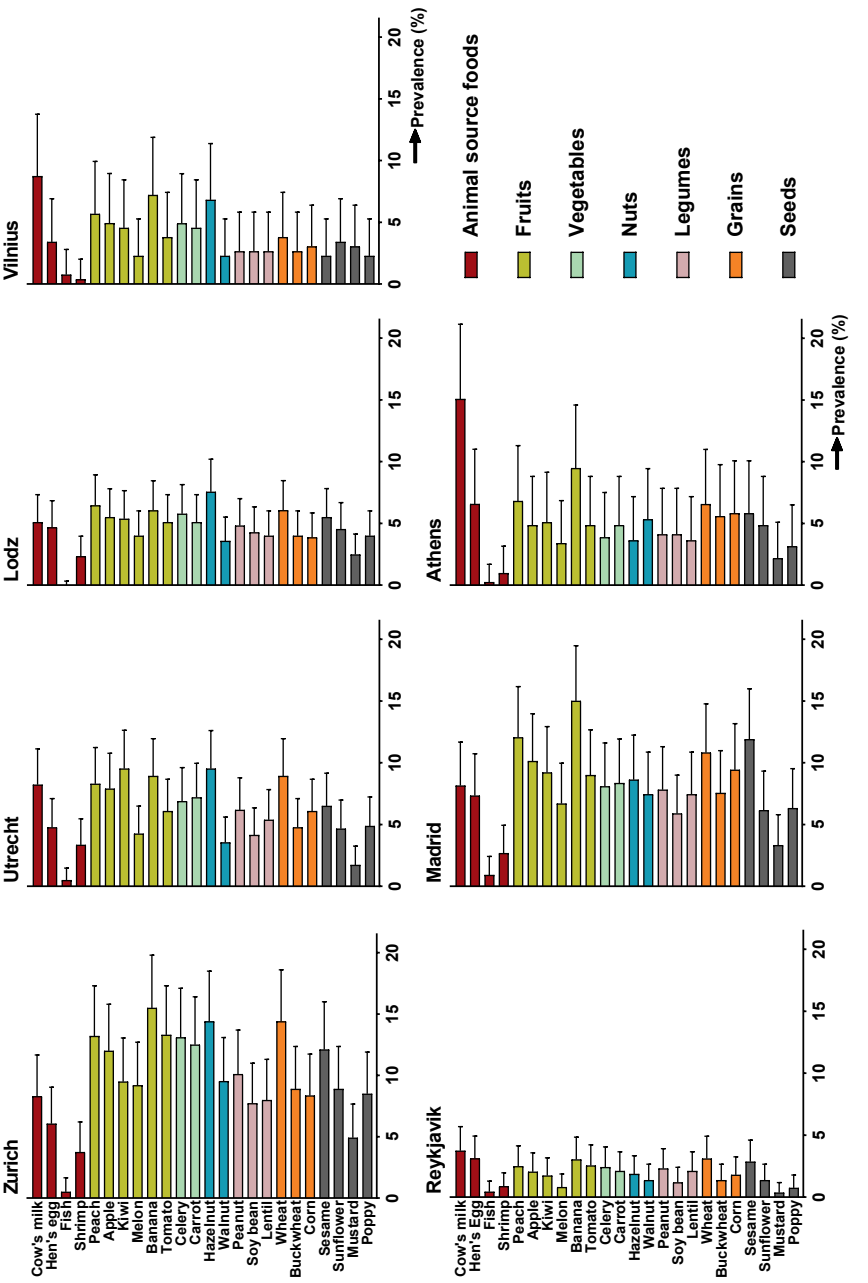

Figure 3. Food sensitisation across Europe
This figure displays the prevalence of FS for each priority food in each centre, and the upper limit of the 95%-CIs. For numeric prevalence estimates of FS, view Table S1. The foods are sorted according to food group. The birch-endemic centres are displayed in the top row.

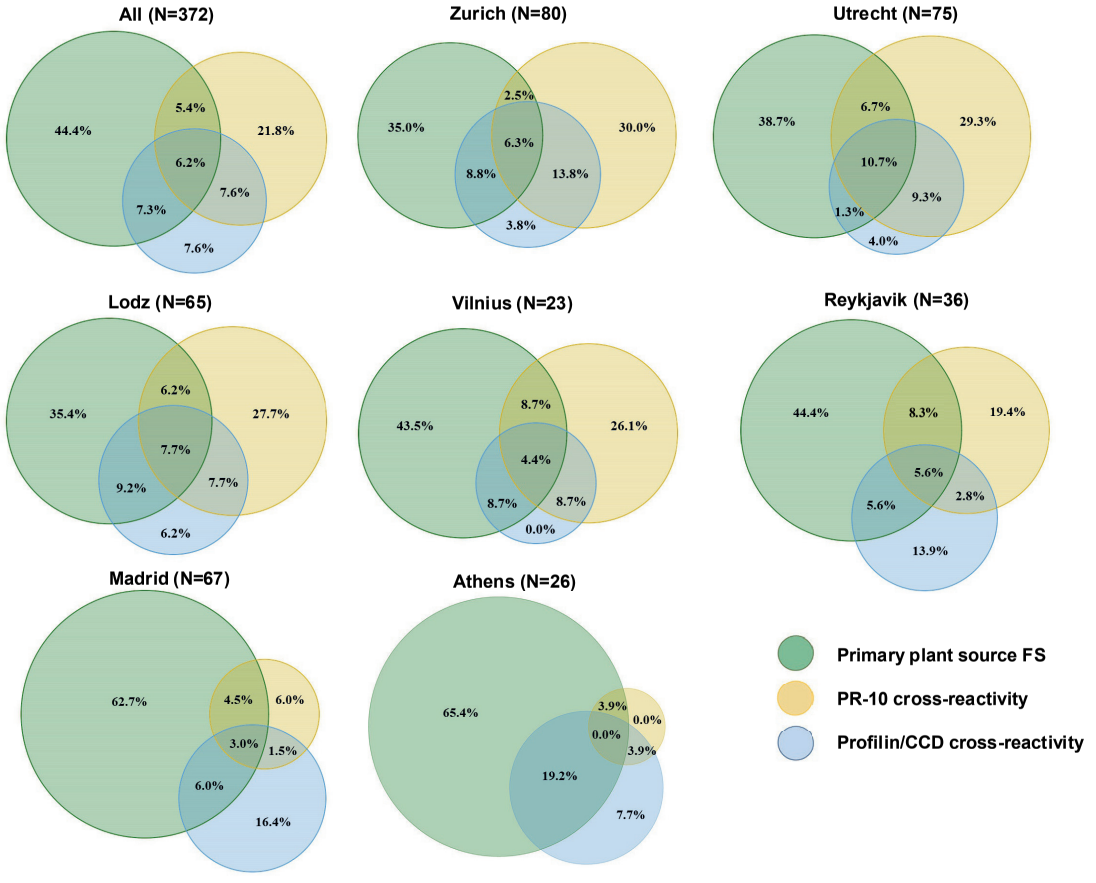

Figure 4. Plant source FS: primary sensitisation and cross-reactivity in children
For classification of primary sensitisation, PR-10, CCD, and profilin cross-reactivity, view Figure S1. Only subjects with sensitisation to plant source foods are included in this figure.

Prevalence of primary FS to all food types (both animal and plant source) in the total study population ranged from 8.6% in Reykjavik to 21.7% in Madrid (Table S1).

Based on component-resolved diagnostics (Figure S1), most food-sensitised children in all centres had primary FS (78.7% of those sensitised), with the highest percentage in Athens (92.5%), followed by Madrid (85.4%), Reykjavik (84.4%), Vilnius (83.3%), Utrecht (76.2%), Lodz (74.4%), and Zurich (67.7%). Relatively, animal source FS was most common in Athens (70.0% of those sensitised) and Reykjavik (60.9%), and least common in Madrid (48.8%) and Zurich (44.1%).

Focusing on subjects with plant source FS, 63.2% of subjects had primary plant source FS, 40.9% plant source FS based on PR-10 cross-reactivity, and 28.5% plant source FS based on profilin or CCD cross-reactivity. Figure 4 shows the overlap between primary plant source FS and cross-reactive plant source FS per centre. Primary plant source FS was most common in Madrid and Athens, PR-10 cross-reactivity occurred most frequently in Utrecht, Zurich, Lodz and Vilnius; and profilin or CCD cross-reactivity occurred in 21.7% to 32.5% of plant source food sensitised subjects in all centres.

Phase II - Probable FA

Prevalence of probable FA was determined from 670 cases with available food serology participating in phase II (Figure 1). Overall, matching food serology was found in 17.2% of all self-reported FAs (Table S2). Probable FA to at least 1 priority food was established in 136 subjects. The prevalence of probable FA was much lower than the prevalence of self-reported FA and of FS, and was found to range from 1.9% in Reykjavik, to 2.0% in Athens, 2.3% in Zurich, 3.0% in Utrecht and Vilnius, 3.9% in Madrid, and 5.6% in Lodz (Figure 2).

Cow's milk, hen's egg, hazelnut, walnut, peanut, lentil, apple, peach, kiwi, banana, carrot, and celery were among the foods most often causing probable FA in the participating centres (Figure 5). Probable FA to cow's milk or hen's egg was relatively common in all centres besides Zurich, where these 2 causative foods were not observed. Hazelnut, apple, carrot and celery probable FAs were prominent in Central and Northern Europe (Zurich, Utrecht, Lodz and Vilnius). Peach and kiwi were important causative foods in most countries, but were particularly dominant in Madrid. Probable FA to peanut was observed everywhere except Vilnius, and made the top 3 in Madrid and Reykjavik. In Athens, unique top causative foods were found compared to the rest of Europe, with walnut, lentils and banana as some of the most common elicitors. Shrimp and fish were important causes of probable FA in Madrid (shrimp and fish), Athens (fish), and Reykjavik (shrimp and fish), but not in the rest of Europe.

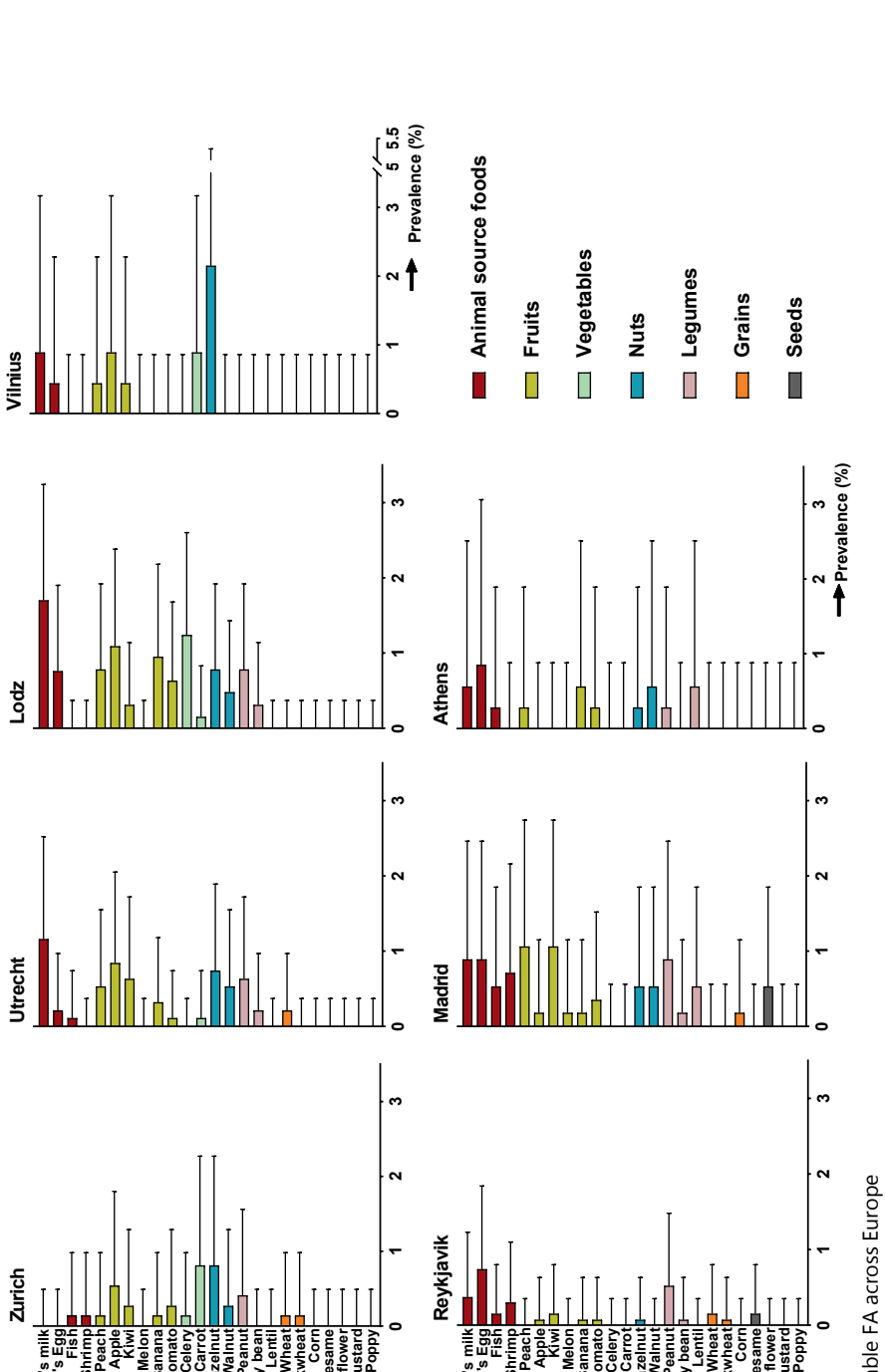

Figure 5. Probable FA across Europe
This figure displays the prevalence of probable FA for each priority food in each centre, and the upper limit of the 95%-CIs. For numeric prevalence estimates of FS, view Table S3. The foods are sorted according to food group. The birch-endemic centres are displayed in the top row.

Regarding symptoms, skin symptoms (80.6%) and oral allergy symptoms (56.0%) were most frequently reported by subjects with probable FA (Table 1). Skin, oral allergy, rhinoconjunctivitis, laryngeal, respiratory and cardiovascular symptoms were reported more often, and reactions occurred more frequently, in subjects with probable FA than in subjects with self-reported FA. Gastrointestinal symptoms were less common in subjects with probable FA.

Phase III - Confirmed FA

DBPCFC was performed in 18 subjects (Figure 1). Table 2 presents the results from challenge testing. Most challenges were performed with shrimp, peanut, hazelnut and apple (N=3 for each food). Overall, 7 of the challenges (38.9%) were positive, 6 (33.3%) negative, and 5 (27.8%) subjects were placebo reactors. The number of challenges performed was too small to obtain reliable values for prevalence of confirmed FA and corresponding symptomatology.

Table 2. Challenge-confirmed FA

Food	Number of challenges*	Reactive	Tolerant	Placebo reactive
Cow's milk	2	0	2	0
Hen's egg	1	1	0	0
Fish	1	0	1	0
Shrimp	3	1	0	2
Peanut	3	2	0	1
Hazelnut	3	2	1	0
Apple	3	0	1	2
Peach	2	1	1	0
Celery	0	0	0	0
Total	18	7	6	5

*One subject was challenged in Athens, 1 in Lodz, 1 in Madrid, 7 in Reykjavik, 5 in Utrecht, 3 in Zurich. No challenges were performed with celery. None of the subjects underwent more than 1 challenge.

Discussion

Summary of findings

The present study reviews the largest available data collection on FA and FS in European school-age children from the general population. It is the first to provide prevalence estimates obtained by uniform methods from socially and climatically varied regions all across Europe. Apparently, 6.5% to 24.6% of 7- to 10-year-old children across Europe report symptoms to at least 1 of 24 foods often implicated in FA (priority foods). A remarkable 11.0% to 28.7% of 7- to 10-year-old children are IgE-sensitised to at least 1 such food. The frequency with which symptoms and IgE sensitisation coincide (i.e. probable FA) is considerably lower, but still impressive at 1.9% to 5.6%. Cow's milk, hen's egg, hazelnut, walnut, peanut, lentil, apple, peach,

kiwi, banana, carrot, and celery were top causative foods for probable FA in the participating countries.

Self-reported FA

With lifetime prevalence estimates of self-reported FA ranging from 13.1% to 45.6% for any food and from 6.5% to 24.6% for priority foods, the current study reveals considerable variation due to geographical location and evaluated foods. The wide range is similar to the 5.7% to 41.8% determined in a systematic review of European studies including children aged 6 to 10 years.[1]

Also comparable between our study and previous literature is that lifetime prevalence of self-reported FA in children appears highest in North-Eastern Europe (Lithuania, Poland), and lowest in South-Eastern Europe (Greece, Turkey);[1] that, overall, cow's milk, fruits, and hen's egg are the most commonly reported foods;[10] and that skin-related and gastrointestinal symptoms are reported most frequently.[10]

Compared with other countries, North-Eastern European countries were found to have particularly high occurrence of self-reported FA to foods not selected as priority foods. Closer inspection of the data revealed that the nonpriority foods most often specified to cause FA were foods with suggested histamine-releasing capacities, such as chocolate, strawberry, and orange.[11]

Food sensitisation

Regarding FS in school-age children, the standardised approach in the current study likely allowed us to obtain more homogenous prevalence estimates from different European regions than a previous systematic review: 11.0% to 28.7% compared with 4.1% to 52.0%.[1] The observed FS patterns in our study correspond with transition from early childhood to adulthood FS patterns. On one hand, cow's milk and hen's egg sensitisation, sources of FA most common in young children,[1] were some of the most prevalent causes of FS in the 7- to 10-year-old children in the current study. On the other hand, non-primary FS based on cross-reactivity with pollen, which is the dominant source of FS in European adults, was also prominent in this age group (Figure S3).[4, 5]

Especially the major PR-10 protein in birch pollen, Bet v 1, is renowned for cross-reacting with certain food allergens in tree nuts, *Rosaceae* fruits, and *Apiaceae* vegetables.[12, 13] PR-10 cross-reactivity likely explains why hazelnut, apple, peach, kiwi, carrot and celery were some of the most common sensitising foods in the birch-endemic countries, Switzerland, the Netherlands, Poland and Lithuania. PR-10 sensitisation was found in 47.8% to 52.2% of plant source food-sensitised children in these countries. In Greece and Spain, only 7.7% and 14.9% of plant source food sensitised subjects had PR-10 sensitisation. Sensitisation to plant source foods like

peach, apple and kiwi in the Mediterranean, is more likely due to primary sensitisation, and partly through lipid transfer protein.[12, 13]

FS based on cross-reactivity with profilin or CCD protein components in pollen (in birch, but also grass, mugwort, and *Parietaria*) was found in 21.7% to 32.5% of food-sensitised subjects. Such cross-reactivity with profilin or CCD goes some way towards explaining the high levels of banana and wheat sensitisation throughout Europe. Of subjects with FS to banana and wheat, respectively 77% and 92% were sensitised to grass, mugwort, *Parietaria* or Bet v 2. Profilin and CCD are known to cause broader cross-reactivity than PR-10 proteins with plant source foods,[14] but FS through profilin does not correspond with symptoms as consistently as FS through PR-10 proteins, and CCD sensitisation is generally thought to be clinically irrelevant.[15, 16] This could help explain the low levels of probable FA to banana and wheat, in contrast to the high levels of FS.

Probable FA

In fact, most food-sensitised subjects did not have concurrent symptoms, and most subjects with self-reported FA appeared not to have an IgE-mediated FA (as viewed in Table S2). Overall, 1.9% to 5.6% of children across Europe were found to have a probable FA. We identified only 1 previous study providing a prevalence estimate for probable FA defined as symptoms and matching IgE sensitisation: 4.6% in 0- to 17-year old children from an unselected paediatric population in Germany.[1, 3] This lack of evidence is rather surprising, because the prevalence of probable FA is a key prevalence estimate in FA epidemiology. Because patients in daily practice tend to decline the time-consuming and burdensome criterion standard of diagnostic testing, oral food challenge, probable FA is often the best attainable end point. This was clearly observed in our study, where too few subjects agreed to undergo DBPCFC to reliably determine the prevalence of challenge-confirmed FA.

Some notably common causes of probable FA were cow's milk, hen's egg, hazelnut, peanut, apple, peach, kiwi, and carrot. Birch pollen-related FA can explain the high prevalence of hazelnut, apple, peach, kiwi and carrot probable FA in countries such as Switzerland, the Netherlands, Poland and Lithuania. In the countries where birch pollen is not a key source of FA (Greece, Spain, and Iceland), animal source foods and other plant source foods appear higher up in the hierarchy of foods most commonly causing probable FA. The low prevalence of cow's milk and hen's egg probable FA in Switzerland was a remarkable finding for which no clear explanation is apparent.

Interestingly, fish and shrimp were the least common sensitising foods across Europe, but they were definitely not the least common causes of probable FA. In literature, fish and shellfish are 2 of the 8 foods suggested to cause most food-allergic

reactions.[17] Apparently, subjects with fish and shrimp sensitisation are likely to have concurrent symptoms. Fish and shrimp were among the top foods causing probable FA in Spain, Greece (for fish) and Iceland. This observation suggests that levels of exposure and frequency of consumption may increase the likelihood of probable FA for certain foods, because seafood consumption is highest in Southern and Northern Europe.[18]

Comparison to adults

The EuroPrevall population study was also conducted in 20- to 54-year-old adults during the same time period, in which the same study design was applied,[6] and the same food and outcome measures were investigated.[4, 5]

One of the major differences between children and adults is observed upon comparison of Figure 4 and Figure S3, which show patterns of cross-reactivity in respectively children and adults sensitised to plant source foods. Where primary FS explains most plant source FS in children, plant source FS due to cross-reactivity dominates in adults, mainly due to birch pollen cross-reactivity in Switzerland, the Netherlands, and Poland.

Despite the relatively more frequent occurrence of cross-reactive FS in adults, overall FS was more prevalent in children than in adults in all countries where both paediatric and adult populations were evaluated (Switzerland, the Netherlands, Spain, Poland, and Iceland). The prevalence of probable FA, however, was lower in children than in adults in Switzerland, the Netherlands, and Spain (>1.5% lower), and similar between children and adults in Poland, Iceland and Greece (<1% difference).[5]

Although the high prevalence estimates of FS compared with probable FA in children may be influenced by high non-response rates, a more likely explanation is an increase in prevalence of FS over time,[19-21] without a parallel increase in symptoms. This theory is supported by recent analyses of longitudinal data from the Isle of Wight Birth Cohort study, where the temporal rise in the prevalence of FS was found to be much more prominent than the rise in the prevalence of FA in children followed from infancy to age 18 years.[22]

Why the prevalence of probable FA is lower in children than in adults in some countries, and not in others, is likely related to the geographical differences in pollen exposure, which plays a role in the prevalence of cross-reactive FS and associated FA (Figure 4 and E3). Both birch pollen- and profilin-related FA occur regularly in adults,[12, 13, 23, 24] and the gap between the percentages of children and of adults demonstrating cross-reactivity with these allergens in Zurich (birch), Utrecht (birch) and Madrid (profilin) may partly explain why probable FA is more common in adults than in children in these countries in particular.

Strengths and limitations

As discussed, a limitation of the present study was the large number of subjects refusing participation in phase III, which prevented acquisition of prevalence estimates for challenge-confirmed FA. It is also important to be aware that the true prevalence estimates of probable FA are likely lower than found in this study. In adults, multiple imputation of missing data from non-responders in phase II revealed that complete case analysis overestimates the prevalence of probable FA, because subjects with FA were more likely to participate in the study.[5] A similar selection bias in our paediatric population cannot be ruled out. Multiple imputation was deemed infeasible because of the high proportion of missing data, and a complete case analysis was preferred. Findings in adults suggest that prevalence estimates of probable FA to any priority food, when all non-responders are included, are 1.5 to 5.5 times lower. For comparison of prevalence estimates in children and adults, unimputed data were used in both cohorts. One should further note that the prevalence of FS and of probable FA focused on 24 foods commonly implicated in FA or frequently consumed in participating countries, and nonpriority foods were not taken into account.

All in all, however, the data analysed for this study are decidedly unique. They are the only pan-European data on FA ever collected according to the same predetermined protocol in a large sample of school-age children from the general population, making valid geographical comparisons possible for the first time. Furthermore, we were able to explore the prevalence of primary FS and cross-reactivity in the general population, and provide previously lacking prevalence estimates of probable FA, a valuable prevalence estimate for daily practice. Finally, because the same study design was applied in adults, the prevalence estimates for children can be compared to those previously published for adults.[5]

Conclusion

In conclusion, a remarkable percentage of 7- to 10-year-old children across Europe appear to be food sensitised, and to a somewhat lesser extent food allergic. Primary and cross-reactive FS, both of which appear clinically relevant in this paediatric age group, occur to varying degrees throughout Europe. Although cow's milk and hen's egg were found to be common causes of probable FA in most countries, the occurrence of reactions to various plant source foods and seafood depends on geographical location, and is clearly related to pollen and, likely, food exposure.

Acknowledgements

We would like to thank all the patients for their participation in the study. We acknowledge the support of the EuroPrevall project by the EU (grant FP6-FOOD-CT-2005-514000).

References

1. Nwaru BI, Hickstein L, Panesar SS, Muraro A, Werfel T, Cardona V, et al. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. Allergy. 2014;69(1):62-75.
2. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy. 2014;69(8):1008-25.
3. Roehr CC, Edenharter G, Reimann S, Ehlers I, Worm M, Zuberbier T, et al. Food allergy and non-allergic food hypersensitivity in children and adolescents. Clin Exp Allergy. 2004;34(10):1534-41.
4. Burney PG, Potts J, Kummeling I, Mills EN, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitisation in European adults. Allergy. 2014;69(3):365-71.
5. Lyons SA, Burney PGJ, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, Clausen M, et al. Food Allergy in Adults: Substantial Variation in Prevalence and Causative Foods Across Europe. J Allergy Clin Immunol Pract. 2019; 7(6):1920-1928.
6. Kummeling I, Mills EN, Clausen M, Dubakiene R, Perez CF, Fernandez-Rivas M, et al. The EuroPrevall surveys on the prevalence of food allergies in children and adults: background and study methodology. Allergy. 2009;64(10):1493-7.
7. Fernandez-Rivas M, Barreales L, Mackie AR, Fritsche P, Vazquez-Cortes S, Jedrzejczak-Czechowicz M, et al. The EuroPrevall outpatient clinic study on food allergy: background and methodology. Allergy. 2015;70(5):576-84.
8. Amlot PL, Kemeny DM, Zachary C, Parkes P, Lessof MH. Oral allergy syndrome (OAS): symptoms of IgE-mediated hypersensitivity to foods. Clin Allergy. 1987;17(1):33-42.
9. Turner PJ, Baumert JL, Beyer K, Boyle RJ, Chan CH, Clark AT, et al. Can we identify patients at risk of life-threatening allergic reactions to food? Allergy. 2016;71(9):1241-55.
10. Steinke M, Fiocchi A, Kirchlechner V, Ballmer-Weber B, Brockow K, Hischenhuber C, et al. Perceived food allergy in children in 10 European nations. A randomised telephone survey. Int Arch Allergy Immunol. 2007;143(4):290-5.
11. Maintz L, Novak N. Histamine and histamine intolerance. Am J Clin Nutr. 2007;85(5):1185-96.
12. Werfel T, Asero R, Ballmer-Weber BK, Beyer K, Enrique E, Knulst AC, et al. Position paper of the EAACI: food allergy due to immunological cross-reactions with common inhalant allergens. Allergy. 2015;70(9):1079-90.
13. Hofmann A, Burks AW. Pollen food syndrome: update on the allergens. Curr Allergy Asthma Rep. 2008;8(5):413-7.
14. Wensing M, Akkerdaas JH, van Leeuwen WA, Stapel SO, Bruijnzeel-Koomen CA, Aalberse RC, et al. IgE to Bet v 1 and profilin: cross-reactivity patterns and clinical relevance. J Allergy Clin Immunol. 2002;110(3):435-42.
15. Hauser M, Roulias A, Ferreira F, Egger M. Panallergens and their impact on the allergic patient. Allergy Asthma Clin Immunol. 2010;6(1):1.
16. McKenna OE, Asam C, Araujo GR, Roulias A, Goulart LR, Ferreira F. How relevant is panallergen sensitisation in the development of allergies? Pediatr Allergy Immunol. 2016;27(6):560-8.
17. Allen KJ, Koplin JJ. The epidemiology of IgE-mediated food allergy and anaphylaxis. Immunol Allergy Clin North Am. 2012;32(1):35-50.
18. Directorate-General for Maritime Affairs and Fisheries (European Commission), EUMOFA. EU consumer habits regarding fishery and aquaculture products, European Commission (January 2017). Available from: https://www.eumofa.eu/documents/20178/84590/EU+consumer+habits_final+report+.pdf/5c61348d-a69c-449e-a606-f5615a3a7e4c, Accessed July 15, 2019.
19. Ezendam J, Klis vdFR, Loveren vH. Time trends in prevalence of sensitisation to milk, egg and peanut in the Netherlands. National Institute for Public Health and the Environment of the Netherlands; 2009. Contract No.: 340350003.

20. Ronmark E, Bjerg A, Perzanowski M, Platts-Mills T, Lundback B. Major increase in allergic sensitisation in schoolchildren from 1996 to 2006 in northern Sweden. J Allergy Clin Immunol. 2009;124(2):357-63, 63 e1-15.
21. Koet LBM, Brand PLP. Increase in atopic sensitisation rate among Dutch children with symptoms of allergic disease between 1994 and 2014. Pediatr Allergy Immunol. 2018;29(1):78-83.
22. Venkataraman D, Erlewyn-Lajeunesse M, Kurukulaaratchy RJ, Potter S, Roberts G, Matthews S, et al. Prevalence and longitudinal trends of food allergy during childhood and adolescence: Results of the Isle of Wight Birth Cohort study. Clin Exp Allergy. 2018;48(4):394-402.
23. Santos A, Van Ree R. Profilins: mimickers of allergy or relevant allergens? Int Arch Allergy Immunol. 2011;155(3):191-204.
24. Rodriguez Del Rio P, Diaz-Perales A, Sanchez-Garcia S, Escudero C, Ibanez MD, Mendez-Brea P, et al. Profilin, a Change in the Paradigm. J Investig Allergol Clin Immunol. 2018;28(1):1-12.

Supplemental files

Supplemental methods on data collection

Questionnaires

To obtain the information required in the EuroPrevall study, specific questions regarding reactions to the 24 priority foods were added to established well-standardised allergy questionnaires.[E1, E2] The phase I screening questionnaire was a one-page document on symptoms and causative foods, which was self-administered. The phase II questionnaire was a more detailed interviewer-administered questionnaire on reactions to all priority foods, medical history and childhood risk factors for FA.

IgE testing

Serum IgE levels to the 24 priority foods and inhalant allergens were measured using commercially available ImmunoCAP tests (Phadia, *currently Thermo Fisher Scientific*, Uppsala, Sweden) in a single laboratory in the Academic Medical Centre, Amsterdam, The Netherlands. All sera that tested positive for at least one of the priority foods, and a random sample of non-sensitised controls, were further tested for sensitisation to specific food allergen components using an allergen microarray assay (component-resolved diagnostics).[E2-4]

DBPCFC

Challenge testing was performed on two separate days following a predefined DBPCFC protocol. [E5] Subjects received increasing doses of either placebo or the concerned food every 20 minutes. The challenge was stopped once subjects had ingested the entire challenge meal, or experienced objective symptoms, or severe subjective symptoms for longer than 45 minutes.

Weighting procedure for population prevalence estimation of probable FA

As described in the methods section, 'probable food allergy' was defined as having symptoms to a specific priority food and sensitisation to the same food. The outcome probable FA was detected amongst the cases in phase I (those with self-reported FA), hereafter termed 'original cases'.

However, some probable food allergies were also found in the controls, i.e. those without self-reported FA to a priority food in phase I who participated in phase II as controls (figure 1). Of these 'newly detected' cases, those who had also reported symptoms (to non-priority foods) during screening were included for prevalence estimation of probable FA. These cases are hereafter termed 'new cases'.

Because of the different sampling fractions (number of subjects with final complete data/ number of subjects approached for participation) for the original cases and the new cases, weighting was necessary to calculate the prevalence of probable FA in the population. The total number of probable food allergies to priority foods was calculated by adding the number of probable food allergies found in the original cases divided by the sampling fraction in the case arm to the number of probable food allergies in the new cases divided by the sampling fraction in the control arm. This total number of probable food allergies was then divided by the total number of responders to phase I to obtain the population prevalence of probable FA. The equation was as follows:

$$\frac{\dfrac{\text{Number of probable FA in original cases}}{\text{Sampling fraction cases}} + \dfrac{\text{Number of probable FA in new cases}}{\text{Sampling fraction controls}}}{\text{Number of responders in phase I}}$$

The prevalence of probable FA to each priority food as estimated in this way was calculated per country**.** As some prevalence estimates were very low, 95%-CIs were calculated after applying a double arscsine transformation. Results were then transformed back to obtain an estimate of the prevalence with 95%-CIs for all probable FA prevalence estimates.

The same method was applied for calculation of prevalence of FS and primary FS. The equation was as follows:

$$\frac{\dfrac{\text{Number of (primary) FS in original cases}}{\text{Sampling fraction cases}} + \dfrac{\text{Number of (primary) FS in controls}}{\text{Sampling fraction controls}}}{\text{Number of responders in phase I}}$$

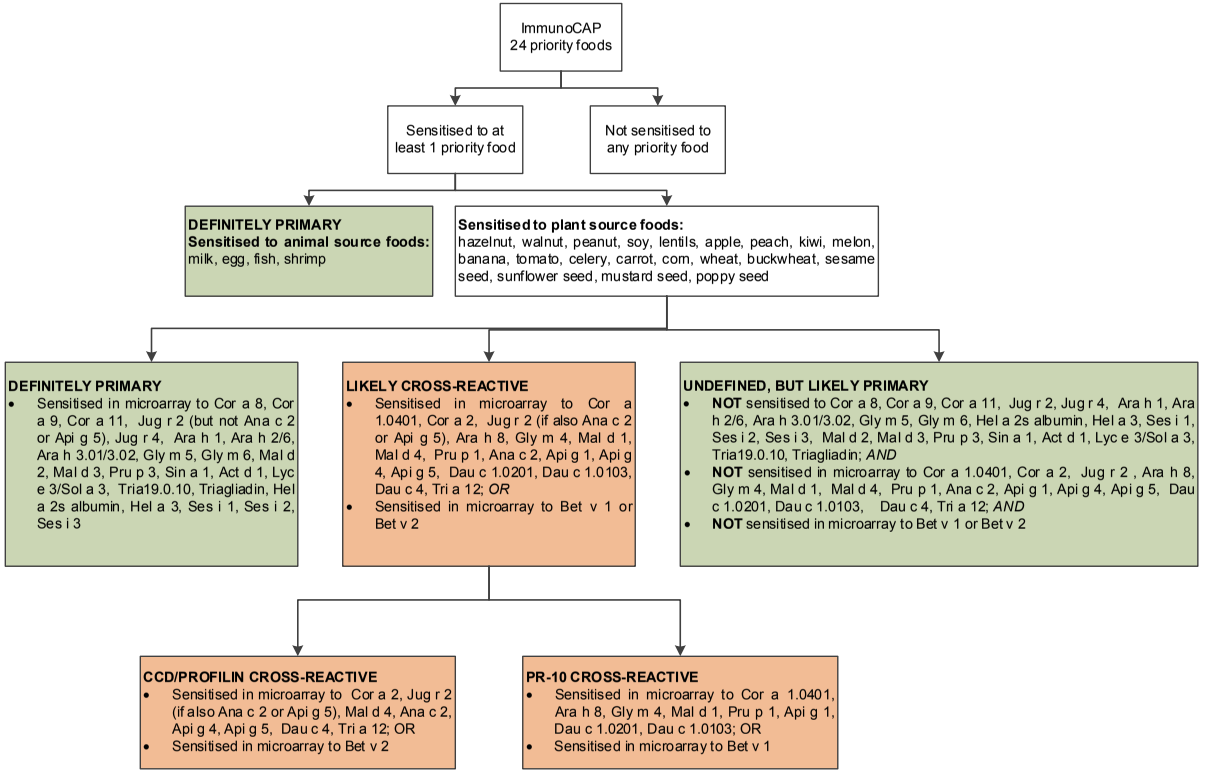

Figure S1. Classification of FS into primary sensitisation and cross-reactive sensitisation
Subjects were classified into 1 *or more* of the boxes depending on their sensitisation patterns (i.e., subjects could have both primary and cross-reactive sensitisation). This is a simplified classification, designed for exploratory purposes, and subjects with cross-reactive sensitisation through food rather than pollen, or with cross-reactive sensitisation to tropomyosins (e.g., shrimp through house-dust mite), have been classified as primary sensitisation. However, aforementioned cross-reactivity patterns are much less common than pollen-related cross-reactivity, and are expected to have only limited influence on the prevalence estimates of primary FS. Green: Primary sensitisation (definitely, or undefined but likely, primary sensitisation). Orange: cross-reactive sensitisation.

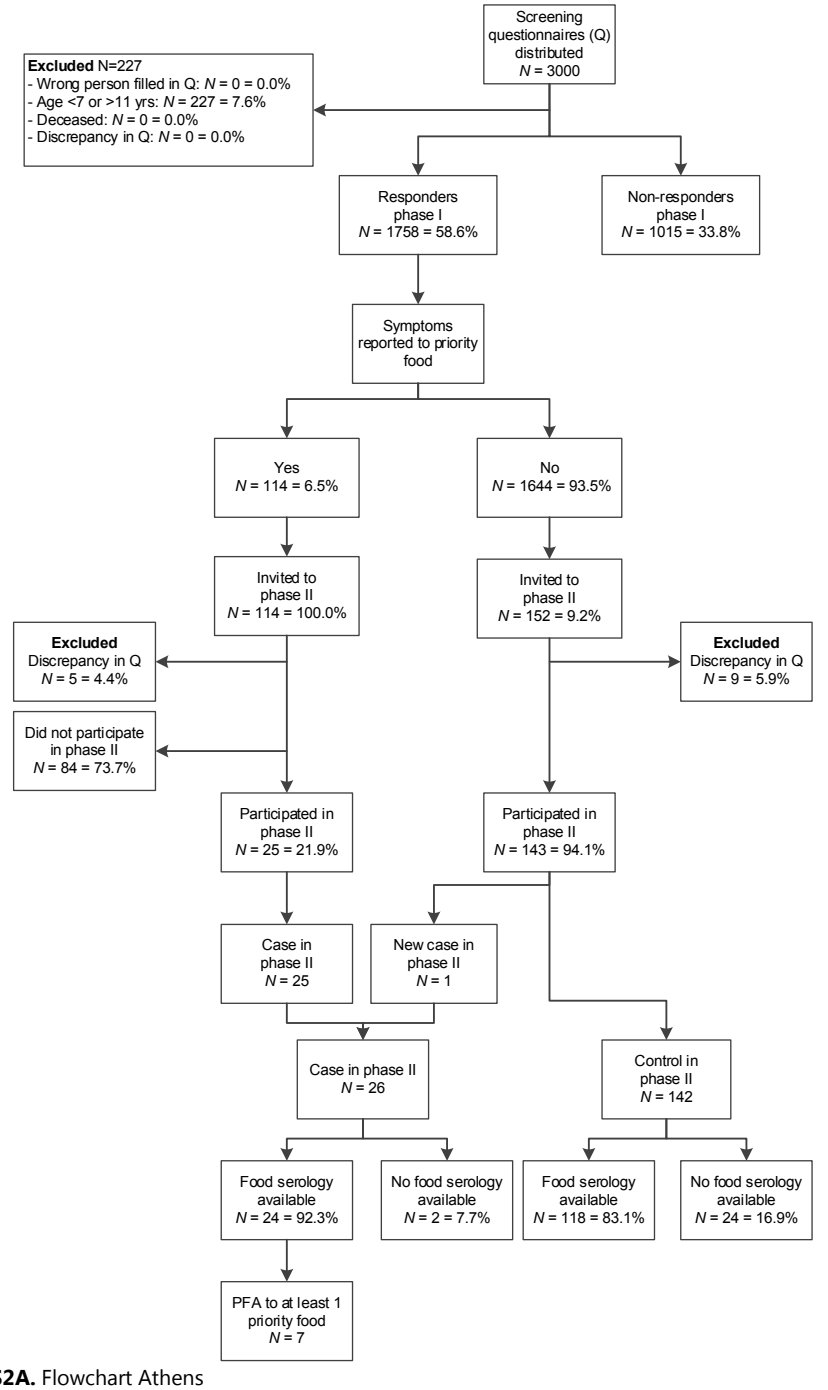

Figure S2A. Flowchart Athens

Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but only symptoms to nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I. *PFA, probable food allergy.*

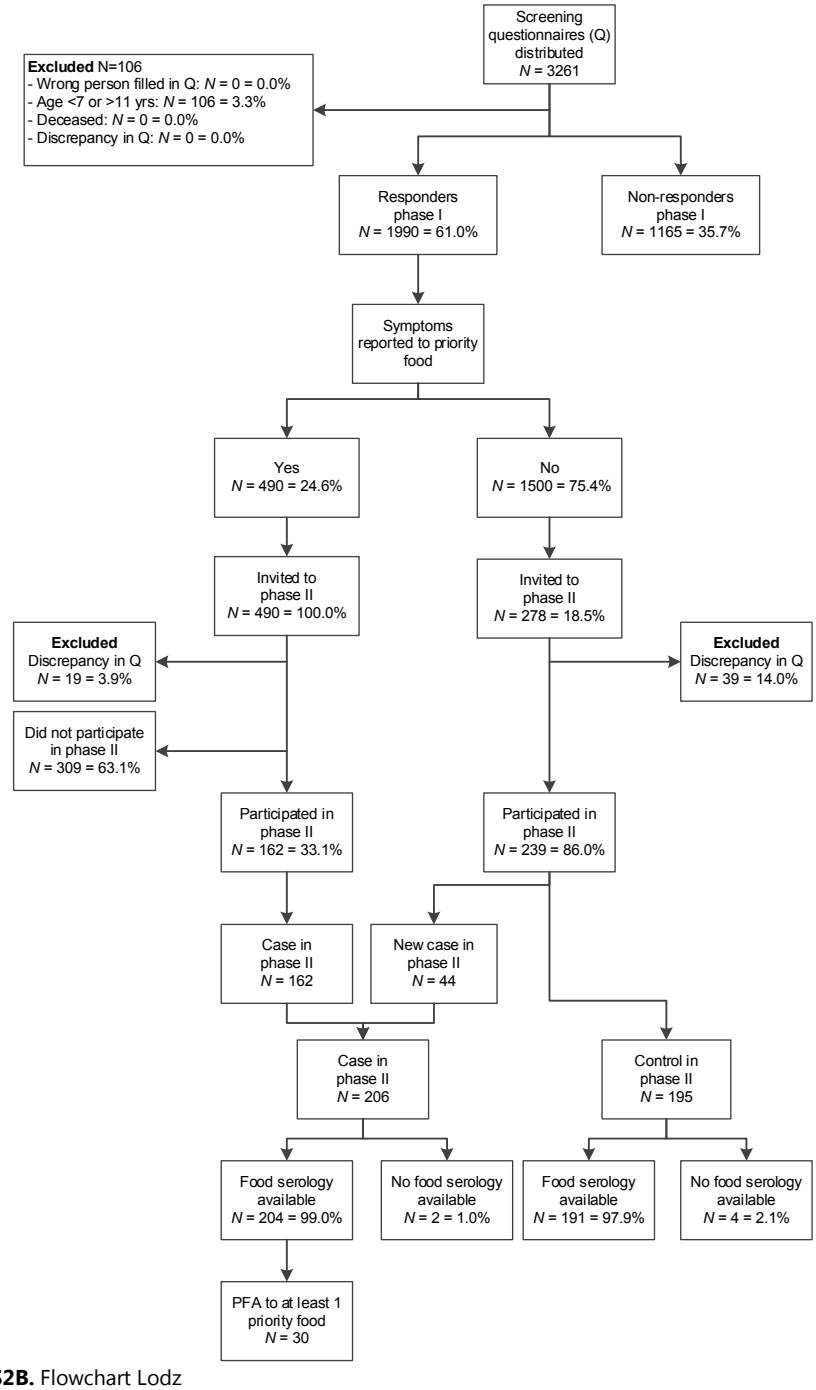

Figure S2B. Flowchart Lodz

Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but only symptoms to nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I. *PFA, probable food allergy.*

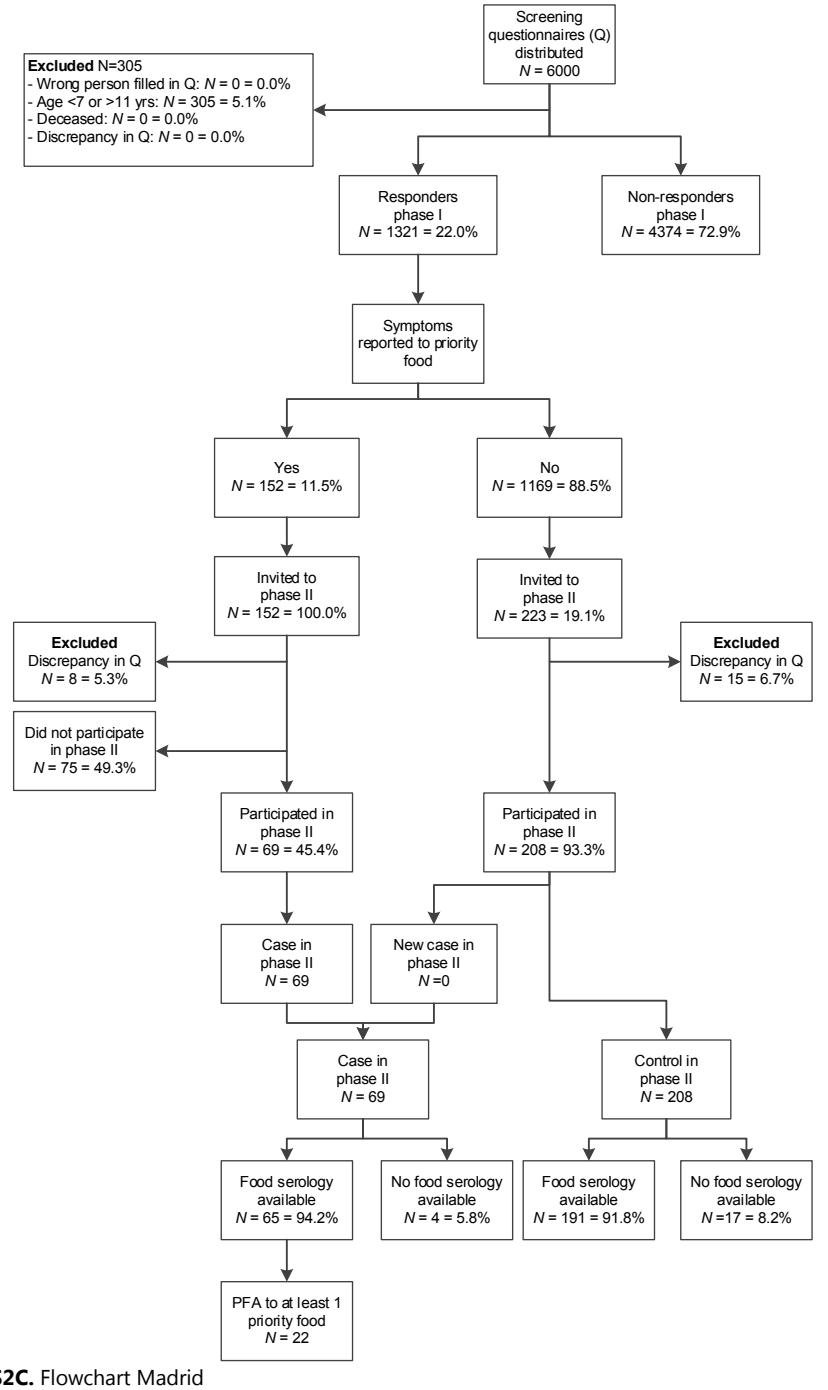

Figure S2C. Flowchart Madrid

Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but only symptoms to nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I. *PFA, probable food allergy.*

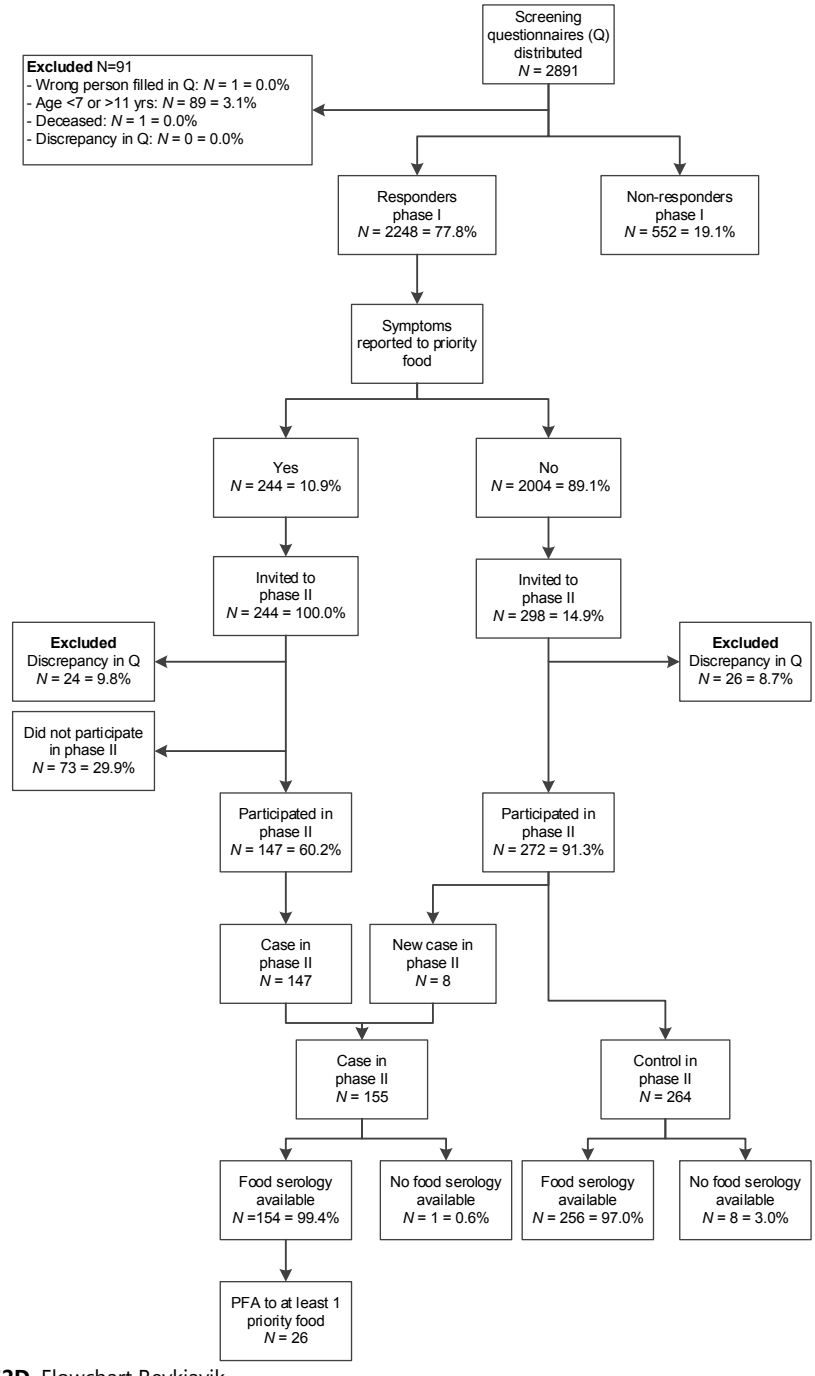

Figure S2D. Flowchart Reykjavik

Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but only symptoms to nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I. *PFA, probable food allergy.*

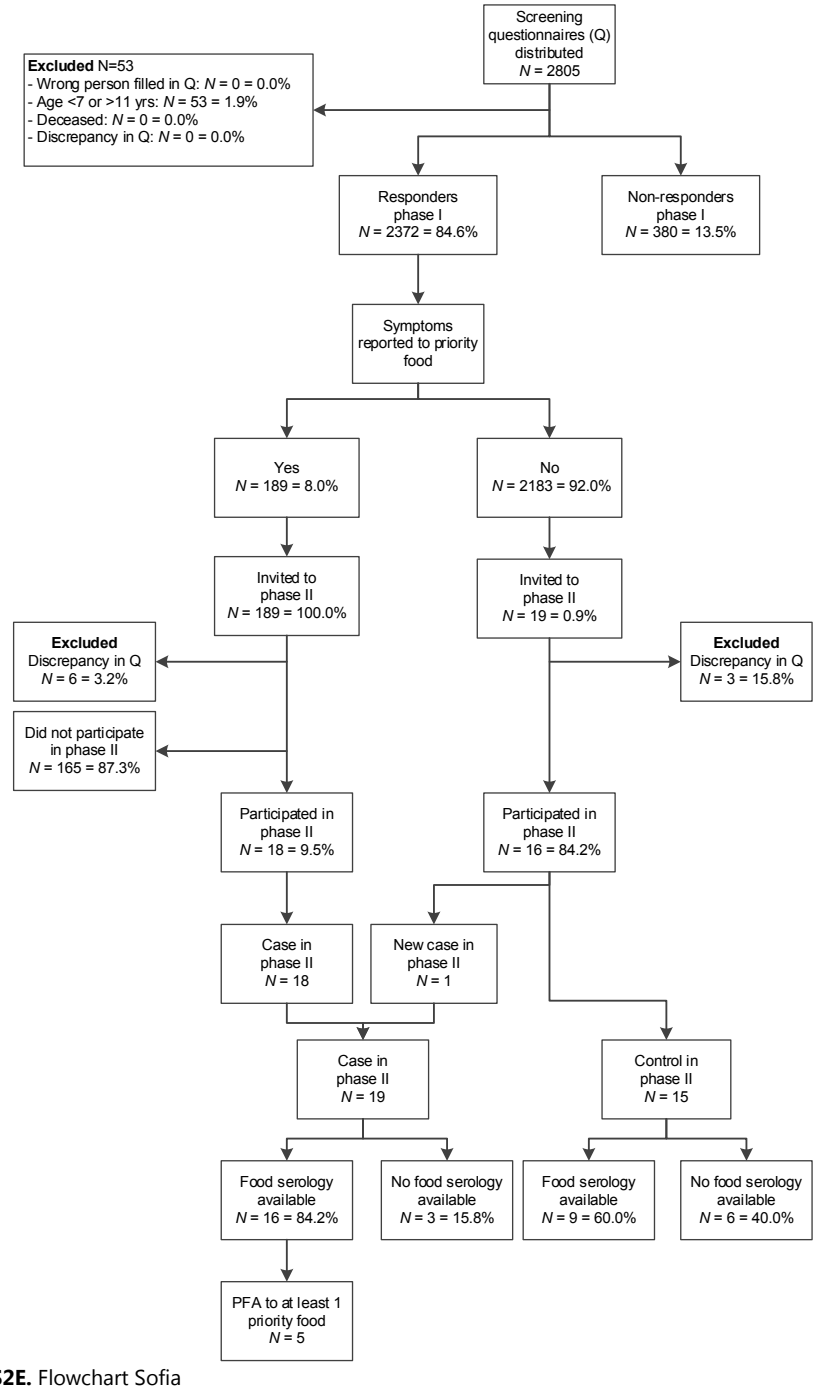

Figure S2E. Flowchart Sofia

Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but only symptoms to nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I. *PFA, probable food allergy.*

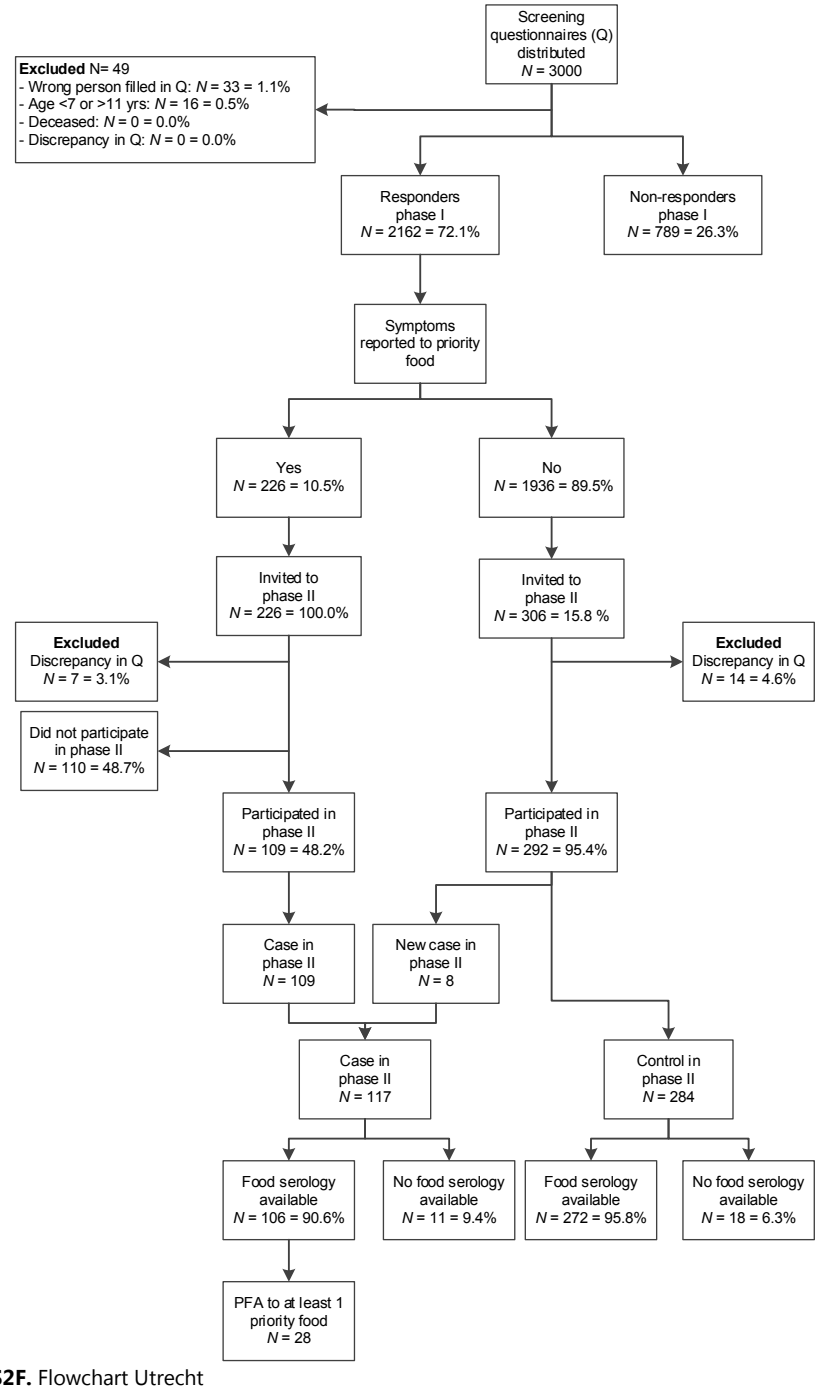

Figure S2F. Flowchart Utrecht

Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but only symptoms to nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I. *PFA, probable food allergy.*

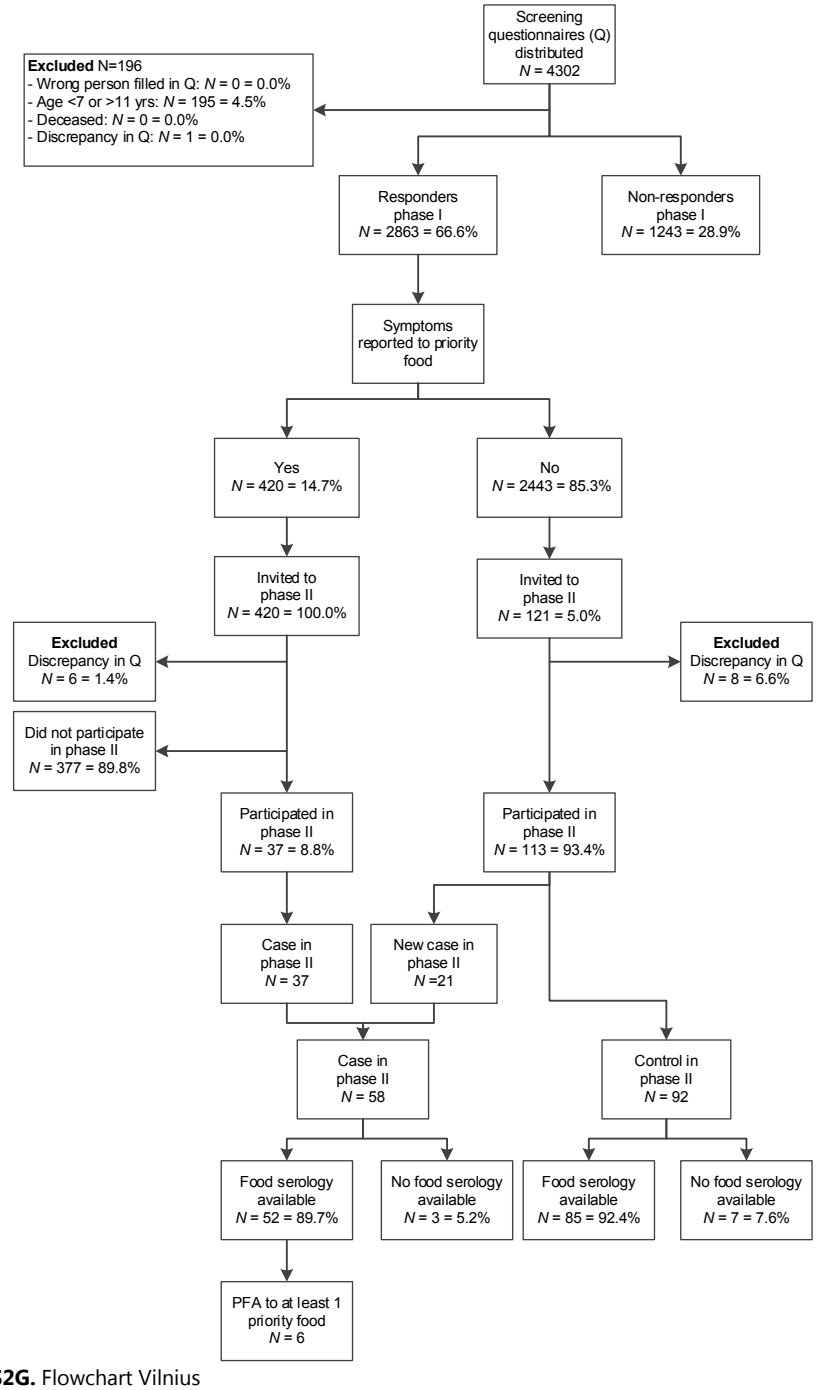

Figure S2G. Flowchart Vilnius

Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but only symptoms to nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I. *PFA, probable food allergy.*

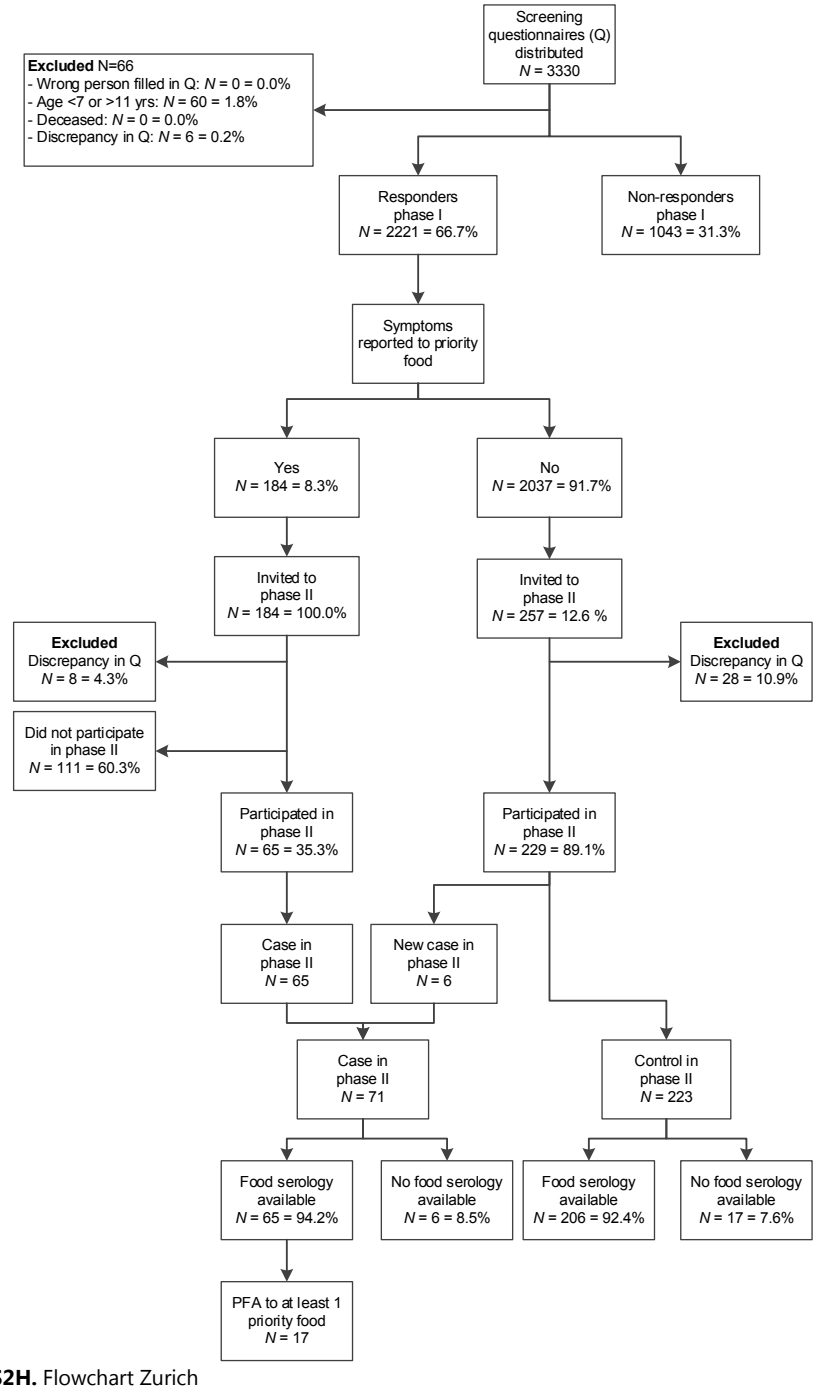

Figure S2H. Flowchart Zurich

Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but only symptoms to nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I. *PFA, probable food allergy.*

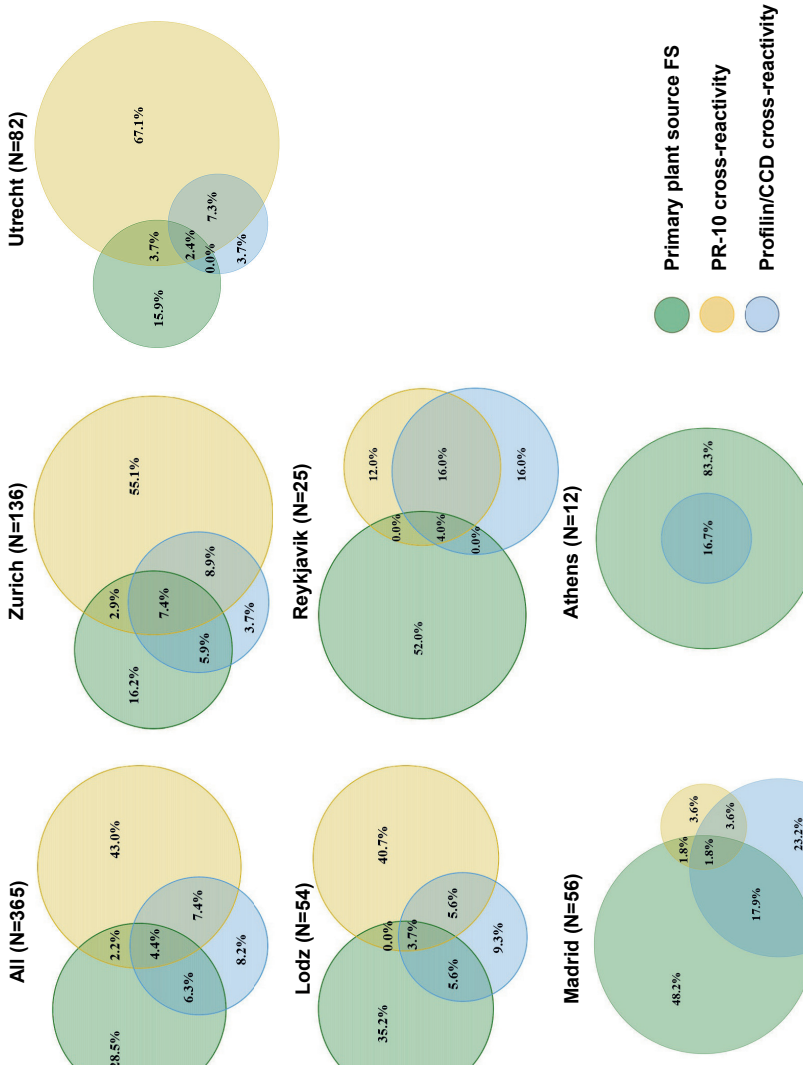

Figure S3. Plant source FS: primary sensitisation and cross-reactivity in adults
For classification of primary sensitisation, PR-10, CCD, and profilin cross-reactivity, view Figure S1.

Table S1. Prevalence of FS per priority food and per centre

Prevalence [95%-CI] of FS:	Zurich	Madrid	Athens	Utrecht	Vilnius	Lodz	Reykjavik
Banana	**15.46 [13.96-16.96]**	**15.01 [13.09-16.94]**	**9.48 [8.11-10.85]**	**8.93 [7.73-10.14]**	**7.21 [6.27-8.16]**	6.05 [5.00-7.10]	3.04 [2.33-3.75]
Wheat	**14.44 [12.98-15.90]**	10.83 [9.15-12.50]	6.56 [5.40-7.71]	**8.93 [7.73-10.14]**	3.79 [3.09-4.49]	**6.06 [5.01-7.10]**	**3.11 [2.39-3.82]**
Hazelnut	**14.35 [12.89-15.81]**	8.63 [7.12-10.15]	3.63 [2.76-4.51]	**9.52 [8.28-10.76]**	**6.82 [5.90-7.74]**	**7.56 [6.40-8.73]**	1.87 [1.31-2.43]
Tomato	13.27 [11.86-14.68]	9.00 [7.46-10.54]	4.85 [3.85-5.86]	6.09 [5.08-7.10]	3.79 [3.09-4.49]	5.09 [4.13-6.06]	2.55 [1.90-3.23]
Peach	13.21 [11.80-14.62]	**12.06 [10.30-13.81]**	**6.81 [5.63-7.98]**	8.30 [7.14-9.47]	5.68 [4.83-6.53]	**6.46 [5.38-7.54]**	2.49 [1.84-3.13]
Celery	13.09 [11.69-14.49]	8.09 [6.62-9.56]	3.88 [2.98-4.79]	6.89 [5.83-7.96]	4.93 [4.13-5.71]	5.78 [4.76-6.81]	2.42 [1.79-3.06]
Carrot	12.46 [11.09-13.83]	8.36 [6.87-9.85]	4.85 [3.85-5.86]	7.20 [7.11-8.29]	4.54 [3.78-5.31]	5.09 [4.13-6.06]	2.11 [1.52-2.71]
Sesame seed	12.10 [10.74-13.45]	**11.90 [10.15-13.64]**	5.82 [4.73-6.92]	6.50 [5.46-7.54]	3.03 [2.40-3.66]	5.50 [4.50-6.51]	2.86 [2.17-3.55]
Apple	11.95 [10.60-13.30]	10.13 [8.50-11.76]	4.85 [3.85-5.86]	7.90 [6.77-9.04]	4.93 [4.14-5.72]	5.50 [4.50-6.50]	2.05 [1.46-2.64]
Peanut	10.06 [8.81-11.31]	7.82 [6.37-9.27]	4.12 [3.19-5.05]	6.18 [5.17-7.20]	2.65 [2.06-3.24]	4.82 [3.87-5.76]	2.31 [1.69-2.93]
Walnut	9.52 [8.30-10.74]	7.45 [6.04-8.87]	5.33 [4.28-6.38]	3.55 [2.77-4.33]	2.28 [1.73-2.82]	3.58 [2.76-4.40]	1.37 [0.89-1.85]
Kiwi	9.49 [8.27-10.71]	9.22 [7.66-10.78]	5.10 [4.08-6.13]	**9.53 [8.29-10.77]**	4.54 [3.78-5.31]	5.37 [4.38-6.36]	1.74 [1.20-2.28]
Melon	9.19 [7.99-10.39]	6.70 [5.35-8.05]	3.40 [2.56-4.25]	4.26 [3.41-5.12]	2.28 [1.73-2.82]	3.99 [3.13-4.86]	0.81 [0.44-1.18]
Buckwheat	8.89 [7.70-10.07]	7.55 [6.13-8.98]	5.58 [4.51-6.66]	4.77 [3.87-5.67]	2.65 [2.06-3.24]	3.99 [3.13-4.85]	1.37 [0.89-1.85]
Sunflower seed	8.89 [7.70-10.07]	6.16 [4.87-7.46]	4.85 [3.85-5.86]	4.67 [3.78-5.56]	3.41 [2.75-4.08]	4.54 [3.63-5.46]	1.37 [0.89-1.85]
Poppy seed	8.50 [7.34-9.66]	6.32 [5.01-7.64]	3.15 [234-3.97]	4.88 [3.97-5.78]	2.28 [1.73-2.82]	3.99 [3.13-4.85]	0.75 [0.39-1.10]
Corn	8.35 [7.20-9.50]	9.43 [7.85-11.01]	5.82 [4.73-6.92]	6.09 [5.08-7.10]	3.04 [2.41-3.67]	3.86 [3.01-4.70]	1.80 [1.25-2.35]
Cow's milk	8.29 [7.14-9.43]	8.15 [6.68-9.63]	**15.08 [13.41-16.75]**	8.22 [7.06-9.37]	**8.74 [7.70-9.11]**	5.09 [4.13-6.06]	**3.74 [2.95-4.52]**
Lentils	7.99 [6.86-9.11]	7.45 [6.04-8.87]	3.63 [2.76-4.51]	5.38 [4.43-6.33]	2.65 [2.06-3.24]	3.99 [3.13-4.85]	2.11 [1.52-2.71]
Soybean	7.72 [6.61-8.83]	5.89 [4.62-7.16]	4.12 [3.19-5.05]	4.15 [3.31-4.99]	2.65 [2.06-3.24]	4.27 [3.38-5.15]	1.19 [0.74-1.63]
Hen's egg	6.06 [5.07-7.06]	7.34 [5.94-8.75]	6.57 [5.41-7.72]	4.77 [3.87-5.67]	3.41 [2.75-4.08]	4.68 [3.75-5.60]	**3.12 [2.40-3.84]**
Mustard seed	4.92 [4.02-5.82]	3.33 [2.36-4.29]	2.18 [1.50-2.86]	1.72 [1.17-2.27]	3.04 [2.41-3.67]	2.48 [1.80-3.16]	0.37 [0.12-0.63]
Shrimp	3.75 [2.96-4.54]	2.68 [1.81-3.55]	0.97 [0.51-1.43]	3.35 [2.59-4.11]	0.38 [0.15-0.60]	2.34 [1.67-3.00]	0.88 [0.49-1.26]
Fish	0.51 [0.21-0.81]	0.91 [0.40-1.42]	0.24 [0.01-0.47]	0.50 [0.21-0.80]	0.76 [0.44-1.08]	0.00 [0.00-0.33]	0.44 [0.17-0.71]
Overall FS	28.73 [26.85 -30.61]	25.78 [23.42-28.14]	23.34 [21.36-25.32]	22.72 [20.96-24.49]	17.84 [16.44-19.24]	16.65 [15.01-18.28]	10.98 [9.69-12.27]
Primary FS	20.26 [18.59-21.94]	21.71 [19.48-23.93]	21.15 [19.24-23.06]	17.17 [15.58-18.76]	15.19 [13.87-16.50]	12.66 [11.20-14.12]	8.62 [7.46-9.78]

Bold indicates the 3 foods most commonly causing FS per centre (more than 3 marked if identical prevalence estimates). Centres are sorted from high to low prevalence of overall FS; foods are sorted from high to low prevalence of FS in Zurich. For prevalence estimates that were close to 0, the double arcsine transformation method was used to prevent obtaining confidence limits with negative values. Subjects with discrepancies in the clinical questionnaires were included in the study population for calculation of prevalence of FS.

Table S2. Percentage of subjects with self-reported FA who had matching FS per priority food

Priority food	Number of subjects with FS*	Number of subjects with self-reported FA*	Number (%) of subjects with self-reported FA that had matching food sensitisation (probable FA)*
Lentils	105	11	5 (45.5)
Apple	152	51	22 (43.1)
Hazelnut	179	69	26 (37.7)
Sunflower seed	104	8	3 (37.5)
Peach	180	48	16 (33.3)
Carrot	143	33	10 (30.3)
Peanut	129	91	26 (28.6)
Celery	147	30	8 (26.7)
Sesame seed	149	8	2 (25)
Banana	188	52	12 (23.1)
Soybean	101	27	6 (22.2)
Shrimp	53	43	9 (20.9)
Walnut	101	74	14 (18.9)
Kiwi	147	104	19 (18.3)
Hen's egg	116	165	26 (15.8)
Buckwheat	109	15	2 (13.3)
Fish	14	74	8 (10.8)
Wheat	165	52	5 (9.6)
Tomato	141	121	10 (8.3)
Cow's milk	149	437	35 (8.0)
Melon	90	15	1 (6.7)
Corn	119	15	1 (6.7)
Mustard seed	50	0	0 (0.0)
Poppy seed	95	0	0 (0.0)
Total	2926	1543	266 (17.2)†

*Source population: 1989 cases and controls participating in phase II with available food serology.
†These 266 probable FAs were found in 136 subjects.

Table S3. Prevalence of probable FA per priority food and per centre

Prevalence [95%-CI] of probable FA:	Lodz	Madrid	Vilnius	Utrecht	Zurich	Athens	Reykjavik
Cow's milk	**1.70 [0.68-3.24]**	**0.89 [0.12-2.46]**	**0.89 [0.01-3.17]**	**1.16 [0.34-2.52]**	0.00 [0.00-0.49]	**0.56 [0.00-2.51]**	**0.37 [0.02-1.23]**
Celery	**1.24 [0.40-2.60]**	0.00 [0.00-0.56]	0.00 [0.00-0.86]	0.00 [0.00-0.37]	0.14 [0.04-0.98]	0.00 [0.00-0.88]	0.00 [0.00-0.35]
Apple	**1.09 [0.32-2.38]**	0.18 [0.02-1.15]	**0.89 [0.01-3.17]**	**0.84 [0.18-2.05]**	**0.54 [0.02-1.80]**	0.00 [0.00-0.88]	0.07 [0.03-0.63]
Banana	0.95 [0.25-2.18]	0.18 [0.02-1.15]	0.00 [0.00-0.86]	0.32 [0.01-1.18]	0.14 [0.04-0.98]	**0.56 [0.00-2.51]**	0.07 [0.03-0.63]
Peanut	0.78 [0.16-1.92]	**0.89 [0.12-2.46]**	0.00 [0.00-0.86]	0.63 [0.09-1.72]	0.41 [0.00-1.56]	0.28 [0.07-1.89]	**0.52 [0.06-1.48]**
Hazelnut	0.78 [0.16-1.92]	0.53 [0.02-1.85]	**2.15 [0.41-5.26]**	**0.74 [0.14-1.89]**	**0.81 [0.10-2.27]**	0.28 [0.07-1.89]	0.07 [0.03-0.63]
Hen's egg	0.76 [0.16-1.90]	**0.89 [0.12-2.46]**	0.44 [0.02-2.28]	0.21 [0.00-0.97]	0.00 [0.00-0.49]	**0.85 [0.01-3.06]**	**0.74 [0.15-1.84]**
Tomato	0.63 [0.10-1.68]	0.35 [0.00-1.52]	0.00 [0.00-0.86]	0.11 [0.02-0.74]	0.27 [0.00-1.29]	0.28 [0.07-1.89]	0.07 [0.03-0.63]
Peach	0.48 [0.05-1.43]	**1.06 [0.19-2.74]**	0.44 [0.02-2.28]	0.53 [0.06-1.55]	0.14 [0.04-0.98]	0.28 [0.07-1.89]	0.00 [0.00-0.35]
Walnut	0.48 [0.05-1.43]	0.53 [0.02-1.85]	0.00 [0.00-0.86]	0.53 [0.06-1.55]	0.27 [0.00-1.29]	**0.56 [0.00-2.51]**	0.00 [0.00-0.35]
Kiwi	0.31 [0.01-1.14]	**1.06 [0.19-2.74]**	0.44 [0.02-2.28]	0.63 [0.09-1.72]	0.27 [0.00-1.29]	0.00 [0.00-0.88]	0.15 [0.01-0.80]
Soybean	0.31 [0.01-1.14]	0.18 [0.02-1.15]	0.00 [0.00-0.86]	0.21 [0.00-0.97]	0.00 [0.00-0.49]	0.00 [0.00-0.88]	0.07 [0.03-0.63]
Carrot	0.15 [0.01-0.83]	0.00 [0.00-0.56]	**0.89 [0.01-3.17]**	0.11 [0.02-0.74]	**0.81 [0.10-2.27]**	0.00 [0.00-0.88]	0.00 [0.00-0.35]
Shrimp	0.00 [0.00-0.37]	0.71 [0.06-2.16]	0.00 [0.00-0.86]	0.00 [0.00-0.37]	0.14 [0.04-0.98]	0.00 [0.00-0.88]	0.30 [0.01-1.10]
Fish	0.00 [0.00-0.37]	0.53 [0.02-1.85]	0.00 [0.00-0.86]	0.11 [0.02-0.74]	0.14 [0.04-0.98]	0.28 [0.07-1.89]	0.15 [0.01-0.80]
Lentil	0.00 [0.00-0.37]	0.53 [0.02-1.85]	0.00 [0.00-0.86]	0.00 [0.00-0.37]	0.00 [0.00-0.49]	**0.56 [0.00-2.51]**	0.00 [0.00-0.35]
Sunflower seed	0.00 [0.00-0.37]	0.53 [0.02-1.85]	0.00 [0.00-0.86]	0.00 [0.00-0.37]	0.00 [0.00-0.49]	0.00 [0.00-0.88]	0.00 [0.00-0.35]
Melon	0.00 [0.00-0.37]	0.18 [0.02-1.15]	0.00 [0.00-0.86]	0.00 [0.00-0.37]	0.00 [0.00-0.49]	0.00 [0.00-0.88]	0.00 [0.00-0.35]
Corn	0.00 [0.00-0.37]	0.18 [0.02-1.15]	0.00 [0.00-0.86]	0.00 [0.00-0.37]	0.00 [0.00-0.49]	0.00 [0.00-0.88]	0.00 [0.00-0.35]
Wheat	0.00 [0.00-0.37]	0.00 [0.00-0.56]	0.00 [0.00-0.86]	0.21 [0.00-0.97]	0.14 [0.04-0.98]	0.00 [0.00-0.88]	0.15 [0.01-0.80]
Buckwheat	0.00 [0.00-0.37]	0.00 [0.00-0.56]	0.00 [0.00-0.86]	0.00 [0.00-0.37]	0.14 [0.04-0.98]	0.00 [0.00-0.88]	0.07 [0.03-0.63]
Sesame seed	0.00 [0.00-0.37]	0.00 [0.00-0.56]	0.00 [0.00-0.86]	0.00 [0.00-0.37]	0.00 [0.00-0.49]	0.00 [0.00-0.88]	0.15 [0.01-0.80]
Mustard seed	0.00 [0.00-0.37]	0.00 [0.00-0.56]	0.00 [0.00-0.86]	0.00 [0.00-0.37]	0.00 [0.00-0.49]	0.00 [0.00-0.88]	0.00 [0.00-0.35]
Poppy seed	0.00 [0.00-0.37]	0.00 [0.00-0.56]	0.00 [0.00-0.86]	0.00 [0.00-0.37]	0.00 [0.00-0.49]	0.00 [0.00-0.88]	0.00 [0.00-0.35]
Overall probable FA	5.60 [3.57-8.11]	3.89 [1.90-6.65]	3.04 [0.85-6.58]	2.96 [1.51-4.93]	2.31 [0.88-4.46]	1.97 [0.36-4.94]	1.93 [0.84-3.52]

Bold indicates the 3 foods most commonly causing probable FA per centre (more than 3 marked if identical prevalence estimates). Centres are sorted from high to low prevalence of overall probable FA; foods are sorted from high to low prevalence of probable FA in Lodz. For prevalence estimates that were close to 0, the double arcsine transformation method was used to prevent obtaining confidence limits with negative values. Subjects with discrepancies in the clinical questionnaires were excluded from the study population for calculation of prevalence of probable FA (because of uncertainties regarding symptoms).

References supplemental files

E1. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. Eur Respir J. 1994;7(5):954-60.

E2. Kummeling I, Mills EN, Clausen M, Dubakiene R, Perez CF, Fernandez-Rivas M, et al. The EuroPrevall surveys on the prevalence of food allergies in children and adults: background and study methodology. Allergy. 2009;64(10):1493-7.

E3. Burney PG, Potts J, Kummeling I, Mills EN, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitisation in European adults. Allergy. 2014;69(3):365-71.

E4. Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, et al. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. FASEB J. 2002;16(3):414-6.

E5. Fernandez-Rivas M, Barreales L, Mackie AR, Fritsche P, Vazquez-Cortes S, Jedrzejczak-Czechowicz M, et al. The EuroPrevall outpatient clinic study on food allergy: background and methodology. Allergy. 2015;70(5):576-84.

Chapter 3

Food allergy in adults: substantial variation in prevalence and causative foods across Europe

J Allergy Clin Immunol Pract. 2019; 7 (6): 1920-1928

Sarah A. Lyons, Peter G.J. Burney, Barbara K. Ballmer-Weber, Montserrat Fernández-Rivas, Laura Barreales, Michael Clausen, Ruta Dubakiene, Cristina Fernández-Pérez, Philipp Fritsche, Monika Jedrzejczak-Czechowicz, Marek L. Kowalski, Tanya Kralimarkova, Ischa Kummeling, Tihomir B. Mustakov, Ans F.M. Lebens, Harmieke van Os-Medendorp, Nikolaos G. Papadopoulos, Todor A. Popov, Alexandros Sakellariou, Paco M.J. Welsing, James Potts, E.N. Clare Mills, Ronald van Ree, André C. Knulst, Thuy-My Le

Abstract

Background
According to the community-based EuroPrevall surveys, prevalence of self-reported food allergy (FA) in adults across Europe ranges from 2% to 37% for any food and from 1% to 19% for 24 selected foods.

Objective
To determine the prevalence of probable FA (symptoms plus specific IgE-sensitisation) and challenge-confirmed FA in European adults, along with symptoms and causative foods.

Methods
In phase I of the EuroPrevall project, a screening questionnaire was sent to a random sample of the general adult population in 8 European centres. Phase II consisted of an extensive questionnaire on reactions to 24 pre-selected commonly implicated foods, and measurement of specific IgE levels. Multiple imputation was performed to estimate missing symptom and serology information for non-responders. In phase III, subjects with probable FA were invited for double-blind placebo-controlled food challenge.

Results
Prevalence of probable FA in adults in Athens, Reykjavik, Utrecht, Lodz, Madrid and Zurich was respectively 0.3%, 1.4%, 2.1%, 2.8%, 3.3% and 5.6%. Oral allergy symptoms were reported most frequently (81.6%), followed by skin symptoms (38.2%) and rhinoconjunctivitis (29.5%). Hazelnut, peach and apple were the most common causative foods in Lodz, Utrecht and Zurich. Peach was also among the top 3 causative foods in Athens and Madrid. Shrimp and fish allergies were relatively common in Madrid and Reykjavik. Of the 55 food challenges performed, 72.8% were classified as positive.

Conclusions
Food allergy shows substantial geographical variation in prevalence and causative foods across Europe. Although probable FA is less common than self-reported FA, prevalence still reaches almost 6% in parts of Europe.

Introduction

Assessing the prevalence of food allergy (FA) is a challenging task. Most studies only investigate the prevalence of self-reported FA.[1-4] Prevalence of self-reported FA is known to be higher than the prevalence of FA defined as symptoms plus specific IgE sensitisation or challenge-confirmed FA,[4,5] on which data are much scarcer.[4,6] For instance, the most recent systematic review focusing on the prevalence of FA according to European studies reports an adult lifetime prevalence of self-reported FA of 9.5% to 35.0%.[4] In comparison, prevalence of symptoms in combination with matching IgE-positivity (probable FA) was addressed in only one study in German adults and found to be 2.2%.[4,7] Prevalence of challenge-confirmed FA was suggested to be as low as 0.9%.[4]

Prevalence estimates also appear to differ remarkably between European countries.[4] It is unknown whether these differences are caused by incomparable study protocols (e.g. different age groups, food types, sampling methods and outcome definitions) or whether these variations really reflect geographical differences in the prevalence of FA. Data from Southern and Eastern Europe are scarce.[4,6,8] To appropriately assess the differences in prevalence of FA across Europe, it is necessary to evaluate countries from all parts of Europe and to use the same study design and outcome definitions in all participating countries.

This was done in the European Union-funded EuroPrevall project, where a standardised protocol was applied in a large population to establish the prevalence of FAs across Europe.[9] In a previous analysis focusing on the prevalence of IgE sensitisation in the adult EuroPrevall population, the prevalence of self-reported FA was briefly evaluated and found to range from less than 2% in Vilnius to 37% in Zurich for any food and from less than 1% in Vilnius and Sofia to 19% in Madrid for 24 commonly implicated foods.[10] In this study, the objective was to thoroughly investigate the prevalence of probable FA (i.e. a combination of self-reported FA and matching IgE sensitisation) and of confirmed FA (diagnosed by double-blind placebo-controlled food challenge (DBPCFC)) in adults across Europe, using the EuroPrevall data. Furthermore, symptoms and causative foods were evaluated.

Methods

Study design

Data for this multi-centre cross-sectional study were collected from 2005 to 2009 as part of the EuroPrevall project, with a case-control study nested within for each centre. The methods have been reported in detail previously.[9,11] In short, a representative sample of 20- to 54-year-old adults was randomly selected from the

general population by 8 European centres, representing different socio-economic and climatic regions across Europe: Zurich (Switzerland), Madrid (Spain), Athens (Greece), Sofia (Bulgaria), Lodz (Poland), Vilnius (Lithuania), Reykjavik (Iceland) and Utrecht (the Netherlands). The study involved 3 phases. In phase I, information regarding adverse reactions to any food, symptoms and incriminated foods was collected in a self-administered 1-page screening questionnaire. For further evaluation including serum IgE measurement, 24 foods that were either known to commonly cause food allergic reactions or thought to be potentially important because of frequent consumption in 1 or more of the participating countries, were determined the so-called *priority foods*. The 24 selected foods were cow's milk, hen's egg, fish, shrimp, peanut, hazelnut, walnut, peach, apple, kiwi, melon, banana, tomato, celery, carrot, corn, lentils, soy, wheat, buckwheat, sesame seed, mustard seed, sunflower seed, and poppy seed. Responders reporting symptoms to 1 of these priority foods (cases) were invited to participate in phase II, along with a random sample of responders who did not report symptoms to any of the priority foods (controls). Phase II consisted of an extensive questionnaire administered by a trained interviewer and serum IgE testing to the priority foods and common inhalant allergens. Additional information was obtained on reactions to all priority foods, allergic comorbidities, and other potential risk factors. Finally, participants with self-reported symptoms and matching IgE to 1 of the 9 priority foods selected for challenge testing (cow's milk, hen's egg, fish, shrimp, peanut, hazelnut, apple, peach, celery) were subjected to phase III, a full clinical evaluation including DBPCFC. Ethical approval was obtained from the local ethical committees of all participating centres, and all participants gave informed consent.

Outcome definitions

Two working definitions of FA were used as outcome measures:

I. Prevalence of probable FA, representing individuals with self-reported FA in combination with matching positive serology (IgE ≥ 0.35 kU_A/l) to at least 1 of the 24 priority foods.

II. Prevalence of confirmed FA, representing individuals with DBPCFC-confirmed FA to at least 1 of the 9 foods selected for challenge testing.

Questionnaires

The questionnaires used in the EuroPrevall study were based on pre-existing well-standardised allergy questionnaires[12] and enriched with specific questions regarding reactions to the selected priority foods.[9]

IgE testing

Commercially available ImmunoCAP tests (Phadia, currently Thermo Fisher Scientific, Uppsala, Sweden) were used to measure serum IgE levels, following the manufacturer's instructions. All serology testing was performed at a single laboratory

in the Amsterdam University Medical Centres, the Netherlands.[9] Specific IgE levels of greater than or equal to 0.35 kU_A/l were considered positive.

DBPCFC

The DBPCFC methods were described in detail elsewhere.[11] DBPCFC was performed on 2 separate days with gradually increasing doses of either the culprit food or placebo with 20 minute intervals. The challenge continued until participants had completely ingested the challenge meal, or had objective symptoms or severe subjective symptoms (e.g. severe itching of palms, soles, head or severe abdominal pain) lasting more than 45 minutes.[11]

Statistical analysis

Prevalence of probable FA was calculated after missing history and serology data in phase II were handled with multiple imputation. Comparison of the cases participating in phase II (*N* = 862) to the cases not participating in phase II (*N* = 803; thus, 48% missing data) suggested that FA prevalence based on the participating cases alone would lead to an overestimation, because participating cases reported more recognisable signs of typical FA. Overall, participating cases reported significantly more frequent reactions than non-participating cases ($p<0.001$). In most countries, there also appeared to be a tendency for participating cases to report more previous doctor-diagnosed FA, oral allergy symptoms, respiratory symptoms, and cardiovascular symptoms, and less gastrointestinal symptoms, than non-participating cases. On the basis of this, we considered data in the non-participating cases likely to be 'missing at random'. Multiple imputation was performed by chained equations.[13,14] Included as predictors in all imputation models were centre, age, sex, all screening variables related to type and frequency of symptoms, previous doctor-diagnosed FA, and a clinical variable indicating occurrence of symptoms to the food corresponding to the respective serology variable. A total of 40 imputations with each 20 iterations were performed.[15,16]

This process resulted in complete data on the presence or absence of symptoms to, and positive or negative serology to, the 24 priority foods for both participating and non-participating cases in phase II. These values were subsequently used to calculate the prevalence of probable FA in each imputed dataset. The calculated rates were weighted back to estimate the population prevalence in each centre according to the sample selection illustrated in the flowcharts (see supplemental 'Weighting procedure for population prevalence estimation of probable FA'; see Figure S1-S8). After double arcsine transformation, which is recommended for stabilising variance when pooling proportions close to 0 or 1,[17] the prevalence estimates were pooled and their 95% confidence intervals (CI) were calculated using Rubin's rules.[14,16,18]

For comparison, prevalence estimates of self-reported FA[10] along with 95%-CIs were reobtained in this study. Sofia and Vilnius were not included in the analysis beyond self-reported FA, because too few cases participated in phase II to justify separate evaluation: 7 cases and 4 cases, respectively. Subjects with evident discrepancies in the questionnaires were also excluded from further analysis (Figure 1).

Self-reported and probable FA were further characterised by determining the absolute number and percentage of subjects with various symptoms and frequencies of reactions. Causative foods were assessed for self-reported FA by calculating the percentage of subjects faulting each specific priority food, and for probable FA by calculating the population prevalence plus 95%-CI for each specific priority food as described above in this section. For confirmed FA, the absolute number and percentage of eligible subjects challenged with each of the selected foods and the frequency of positive challenge tests were determined.

Analyses were conducted with SPSS version 25 and R version 3.4.1.

Results

Phase I – Self-reported FA

The flowcharts of the study are depicted in Figure 1 and E1-E8. Response rates per centre and prevalence estimates of self-reported FA were published previously by Burney *et al*, the latter of which are shown in Figure 2 for comparison with the prevalence of probable FA.[10] In subjects with self-reported FA, the most commonly reported causative priority foods were cow's milk (7.8%), apple (5.6%), tomato (4.3%), hen's egg (4.2%), kiwi (3.8%), shrimp (3.5%), fish (3.1%), hazelnut (3.0%), walnut (2.2%), wheat (2.2%) and peanut (2.0%).

Subjects with a self-reported allergy to any food most often reported either gastrointestinal symptoms (44.5%) or skin symptoms (38.8%) (Table 1). Oral allergy symptoms were reported by 34.8%. For subjects with a self-reported allergy to priority foods, oral allergy symptoms (46.9%), skin symptoms (42.5%) or gastrointestinal symptoms (41.1%) were the most commonly mentioned. Alternatively, 18.8% of subjects with self-reported allergy to any food and 20.5% of subjects with self-reported allergy to priority foods reported respiratory or cardiovascular symptoms.

Phase II - Probable FA

For prevalence of probable FA, a sample of 693 cases and 1355 controls was studied. Of the subjects in the control arm, 12.5% (169 of 1355) reported symptoms to priority foods in phase II in addition to symptoms to only non-priority foods in phase I (Figure 1).

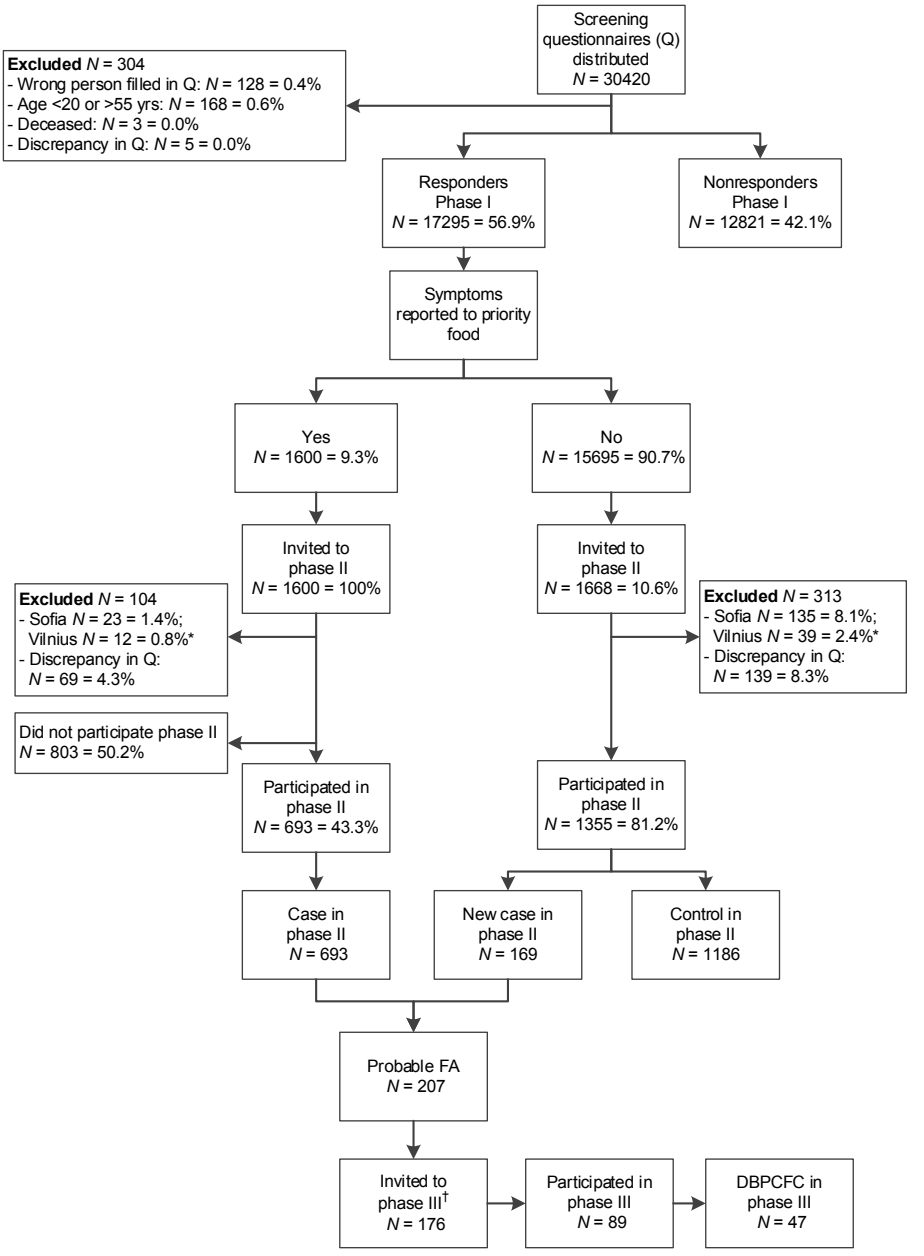

Figure 1. Flowchart

Overall participation in phase I, II and III of the EuroPrevall population-based study in adults. Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but symptoms to only nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I. *Sofia and Vilnius were excluded from calculation of probable FA prevalence because of lack of cases participating in phase I. †Probable FA to cow's milk, hen's egg, fish, shrimp, peanut, hazelnut, apple, peach, or celery. *DBPCFC, double-blind placebo-controlled food challenge.*

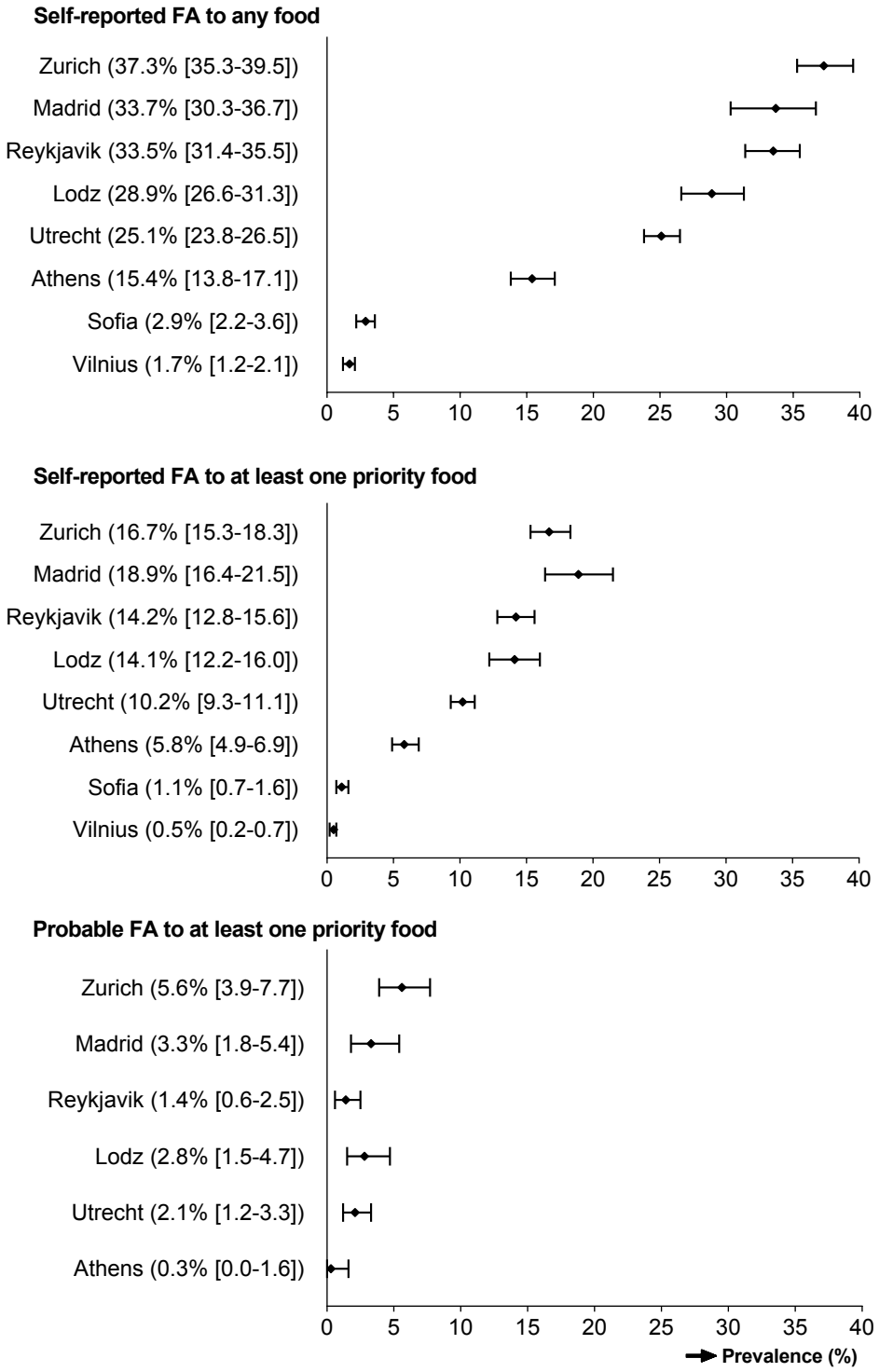

Figure 2. Prevalence of probable FA compared with prevalence of self-reported FA

Table 1. Reported symptoms for self-reported and probable FA

	Self-reported FA to any food (N=3657)	**Self-reported FA to priority food (N=1600)**	**Probable FA to priority food (N=207)**
Age in years, *median (IQR*)	36.6 (29.0-44.9)	36.9 (29.2-44.8)	35.4 (28.8-42.6)
Female sex	2286 (62.8)	1057 (66.3)	114 (55.1)
Oral allergy symptoms	1203 (34.8)	725 (46.9)	169 (81.6)
Skin symptoms	1340 (38.8)	649 (42.5)	79 (38.2)
Rhinoconjunctivitis	686 (20.2)	367 (24.4)	61 (29.5)
Gastrointestinal symptoms	1550 (44.5)	630 (41.1)	52 (25.1)
Difficulty swallowing	350 (10.3)	192 (12.8)	49 (23.7)
Respiratory symptoms	407 (12.0)	212 (14.1)	44 (21.3)
Cardiovascular symptoms	333 (9.8)	145 (9.7)	15 (7.2)
Other symptoms	1466 (43.0)	647 (42.9)	61 (29.5)
Lifetime frequency of reactions			
1x	645 (18.1)	183 (11.7)	9 (4.4)
2-4x	963 (27.0)	361 (23.0)	34 (16.7)
>4x	1954 (54.8)	1025 (65.3)	161 (78.9)
Previous doctor-diagnosis of FA	676 (18.9)	418 (26.6)	79 (38.7)

Values are N (%) unless otherwise indicated. Oral allergy symptoms: itching/tingling/swelling of the mouth/lips/throat. Skin symptoms: rash/nettle sting/itchy skin. Rhinoconjunctivitis: runny/stuffy nose or red/sore/running eyes. Gastrointestinal symptoms: diarrhoea/vomiting. Respiratory symptoms: breathlessness. Cardiovascular symptoms: fainting/dizziness. Other symptoms: stiffness in joints or headaches or other symptoms. *IQR, interquartile range.*

This was most likely due to limitations in space for reporting foods in the phase I questionnaire, whereas in phase II, subjects were asked whether they had ever experienced symptoms to each of the 24 priority foods separately. These 169 subjects were defined as 'new cases' and analysed along with the 693 original cases, leading to a total of 862 cases. Cases had a median age of 37.3 years and 65.7% were females; controls had a median age of 38.9 years and 50.9% were females.

Of the 862 cases participating in phase II, 207 were found to have probable FA (Figure 1). The weighted population prevalence of probable FA is presented per centre and per food in Figure 2 and Table 2 for the 6 centres for which we could calculate the prevalence. The given prevalence estimates are the results from multiple imputation, which included estimation of probable FA in subjects who did not participate in phase II. These prevalence estimates were a factor 1.5 to 5.5 lower than those based on analysis without imputation (complete case analysis, Table S1). This indicates that prevalence estimates without adjusting for non-response (i.e. without imputation) could lead to overestimation of the prevalence.

Table 2. Prevalence of probable FA per priority food and per centre

Prevalence [95%-CI] of probable FA to :	Zurich	Madrid	Reykjavik	Lodz	Utrecht	Athens
At least 1 priority food	5.64 [3.94-7.66]	3.28 [1.78-5.35]	1.36 [0.57-2.54]	2.84 [1.51-4.65]	2.12 [1.19-3.35]	0.31 [0.02-1.65]
Hazelnut	**2.57 [1.47-4.02]**	0.76[0.16-1.96]	0.10 [0.00-0.60]	**1.33 [0.49-2.66]**	**0.93 [0.36-1.81]**	0.06 [0.19-0.99]
Peach	**2.02 [1.08-3.30]**	**1.61 [0.63-3.18]**	0.00[0.00-0.28]	**0.45 [0.05-1.35]**	**0.60 [0.17-1.33]**	**0.10 [0.00-1.14]**
Apple	**1.90 [0.99-3.15]**	0.57 [0.08-1.65]	0.05 [0.02-0.48]	**0.75 [0.17-1.83]**	**0.91 [0.34-1.77]**	0.00 [0.00-0.68]
Kiwi	1.34 [0.60-2.42]	0.64 [0.11-1.77]	0.31 [0.02-0.99]	0.30 [0.01-1.09]	0.57 [0.15-1.29]	0.00 [0.00-0.68]
Carrot	1.01 [0.39-1.97]	0.81 [0.18-2.03]	**0.38 [0.05-1.12]**	0.15 [0.00-0.80]	0.23 [0.01-0.76]	0.00 [0.00-0.68]
Walnut	0.58 [0.11-1.45]	0.71 [0.14-1.88]	0.05 [0.02-0.48]	0.15 [0.00-0.80]	0.10 [0.00-0.51]	**0.29 [0.02-1.61]**
Melon	0.46 [0.09-1.17]	**0.95 [0.25-2.24]**	0.10 [0.00-0.60]	0.00 [0.00-0.36]	0.05 [0.01-0.39]	0.00 [0.00-0.68]
Shrimp	0.37 [0.05-1.04]	**0.82 [0.19-2.04]**	**0.36 [0.04-1.08]**	0.35 [0.02-1.18]	0.36 [0.06-0.97]	0.00 [0.00-0.68]
Peanut	0.37 [0.02-1.17]	0.45 [0.05-1.45]	0.00 [0.00-0.28]	0.35 [0.02-1.18]	0.05 [0.01-0.39]	0.02 [0.00-0.82]
Celery	0.24 [0.01-0.81]	0.00 [0.00-0.44]	0.33 [0.03-1.03]	0.07 [0.02-0.63]	0.03 [0.03-0.32]	0.00 [0.00-0.68]
Cow's milk	0.24 [0.00-1.02]	0.18 [0.00-0.97]	0.00 [0.00-0.28]	0.12 [0.01-0.74]	0.00 [0.00-0.21]	0.00 [0.00-0.68]
Wheat	0.19 [0.00-0.73]	0.37 [0.02-1.31]	0.10 [0.00-0.60]	0.00 [0.00-0.36]	0.05 [0.01-0.39]	0.00 [0.00-0.68]
Lentil	0.16 [0.07-0.93]	0.00 [0.00-0.44]	0.00 [0.00-0.28]	0.00 [0.00-0.36]	0.03 [0.03-0.32]	0.00 [0.00-0.68]
Tomato	0.10 [0.00-0.55]	0.15 [0.01-0.90]	0.15 [0.00-0.71]	0.30 [0.01-1.10]	0.08 [0.01-0.46]	0.00 [0.00-0.68]
Soybean	0.08 [0.02-0.56]	0.00 [0.00-0.44]	0.00 [0.00-0.28]	0.00 [0.00-0.36]	0.03 [0.03-0.32]	0.00 [0.00-0.68]
Sunflower seed	0.05 [0.02-0.42]	0.00 [0.00-0.44]	0.00 [0.00-0.28]	0.00 [0.00-0.36]	0.00 [0.00-0.21]	**0.07 [0.17-1.05]**
Banana	0.05 [0.02-0.42]	0.04 [0.00-0.65]	**0.49 [0.08-1.29]**	0.00 [0.00-0.36]	0.05 [0.01-0.39]	0.00 [0.00-0.68]
Corn	0.05 [0.02-0.42]	0.00 [0.00-0.44]	0.05 [0.02-0.48]	0.00 [0.00-0.36]	0.00 [0.00-0.21]	0.00 [0.00-0.68]
Sesame seed	0.03 [0.00-0.37]	0.00 [0.00-0.44]	0.00 [0.00-0.28]	0.00 [0.00-0.36]	0.00 [0.00-0.21]	0.00 [0.00-0.68]
Fish	0.02 [0.00-0.35]	0.44 [0.04-1.44]	0.15 [0.00-0.71]	0.00 [0.00-0.36]	0.08 [0.01-0.46]	0.00 [0.00-0.68]
Hen's egg	0.00 [0.00-0.25]	0.06 [0.00-0.70]	0.21 [0.00-0.81]	0.31 [0.01-1.11]	0.00 [0.00-0.21]	0.02 [0.00-0.84]
Mustard seed	0.00 [0.00-0.25]	0.00 [0.00-0.44]	0.00 [0.00-0.28]	0.03 [0.00-0.52]	0.00 [0.00-0.21]	0.00 [0.00-0.68]
Buckwheat	0.00 [0.00-0.25]	0.00 [0.00-0.44]	0.00 [0.00-0.28]	0.00 [0.00-0.36]	0.00 [0.00-0.21]	0.00 [0.00-0.68]
Poppy seed	0.00 [0.00-0.25]	0.00 [0.00-0.44]	0.00 [0.00-0.28]	0.00 [0.00-0.36]	0.00 [0.00-0.21]	0.00 [0.00-0.68]

Bold indicates the 3 foods most commonly causing probable FA per centre. Centres are sorted from high to low prevalence of overall probable FA; foods are sorted from high to low prevalence of FS in Zurich. *CI, confidence interval.*

The prevalence of probable FA to at least one priority food was much lower than prevalence of self-reported FA and ranged from 0.3% [95%-CI 0.0-1.7] to 5.6% [95%-CI 3.9-7.7] (Figure 2). Of subjects with self-reported FA to a priority food, matching IgE sensitisation was found for 17.9%. Positive serology was mostly found if symptoms to apple, peach, carrot or hazelnut were reported, in 46.8-60.0% of cases. Positive serology was found in 0.5% or less when subjects reported symptoms to poppy seed, sesame seed, mustard seed, or cow's milk. The centres with the highest prevalence of probable FA were the same as those with the highest prevalence of self-reported FA: Zurich and Madrid. The lowest prevalence was found in Reykjavik and Athens. Hazelnut, apple and peach were the foods most frequently causing probable FA in Zurich, Lodz and Utrecht (Table 2). Probable FA to peach was also found frequently in the Mediterranean centres, Madrid and Athens. Shrimp was most commonly reported in Madrid, but was also one of the most important causative foods in Reykjavik. Probable fish allergy was most frequently seen in Madrid and Reykjavik.

Compared with self-reported FA, oral allergy symptoms, difficulty swallowing, rhinoconjunctivitis and respiratory symptoms were more common in subjects with probable FA (Table 1). Oral allergy symptoms were the most frequently reported symptoms for subjects with a probable FA in all included centres (81.6%, Table 1) and ranged from 60.0% of subjects in Athens to 93.3% in Utrecht. In contrast, gastrointestinal symptoms were less frequently reported in subjects with probable FA than in subjects with self-reported FA. Although time until onset of symptoms was not available for all foods, 95.8% of subjects classified as having probable FA to a priority food and naming that same food as causing their most severe reaction, reported reacting within 2 hours of ingestion.

Phase III – Confirmed FA

Of the 207 subjects with probable FA to a priority food in phase II, 176 patients reported symptoms to 1 of the 9 priority foods selected for challenge testing. A total of 89 subjects with probable FA to 1 of these priority foods (50.6%) proceeded to phase III of the study, where 55 challenges were finally performed in 47 individuals who agreed to the procedure (29 from Zurich, 12 from Utrecht, 4 from Madrid, 1 from Lodz and 1 from Athens). These 47 challenge-tested subjects had a median age of 35.5 years and 55.3% were females.

There were no significant differences in age, sex, level of education, or reported symptoms between the 47 subjects who underwent DBPCFC in phase III and the 129 subjects who declined further evaluation at a Bonferroni-corrected p-value of 0.004. Details of the challenge results are presented in Table 3. Most challenges were performed for hazelnut (n=28), apple (n=12) and peach (n=9). No challenges were performed for fish, cow's milk or hen's egg. The percentage of positive challenges to

foods for which at least 5 challenges were performed ranged from 55.6% for peach to 82.1% for hazelnut. Overall, 72.7% (40 of 55) of the challenge tests were classified as reactive. Too few challenges were performed to estimate the prevalence of confirmed FA or report on symptoms.

Table 3. Challenge-confirmed FA

Food	Number of challenges*	Reactive	Tolerant	Placebo reactive
Hazelnut	28	23	3	2
Apple	12	8	1	3
Peach	9	5	2	2
Peanut	3	2	0	1
Celery	2	1	1	0
Shrimp	1	1	0	0
Total	55	40	7	8

*One subject was challenged in Athens, 1 in Lodz, 4 in Madrid, 12 in Utrecht, 29 in Zurich. No challenges were performed for cow's milk, hen's egg or fish. Six subjects underwent challenge with 2 foods, and 1 subject underwent challenge with 3 foods.

Discussion

The standardised methodology used all across Europe for the first time in the EuroPrevall project provided the opportunity to conclude reliably that prevalence of FA shows considerable geographical variation and depends strongly on the chosen definition. Compared with previously presented prevalence estimates of self-reported FA,[10] probable FA was less common, with the highest prevalence found to be 5.6% in Switzerland, followed by Spain, Poland, the Netherlands, Iceland and finally Greece at 0.3%. Causative foods differed between countries, with plant foods dominating. Challenge testing was positive in around 73% of subjects with probable FA who underwent DBPCFC.

This is the first study extensively addressing probable FA in multiple European countries and showing its prevalence to range from 0.3 to 5.6%. Systematic review in Europe identified only 1 article between 2002 and 2012 reporting probable FA in 2.2% of German adults.[4] Although FA diagnosed by DBPCFC is considered the gold standard, many patients decline to undergo this burdensome diagnostic process, as can be recognised in the large number of subjects unwilling to proceed to phase III of our study (Figure 1). In daily practice, diagnosis of FA is often based on the combination of symptoms and IgE sensitisation,[8] which underlines the clinical relevance of probable FA and the value of prevalence estimates for FA defined as such.

The prevalence of probable FA after multiple imputation (0.3%-5.6%) was lower than with complete case analysis (1.7%-8.4%). Previous studies on FA prevalence in Europe

did not perform multiple imputation for non-responders. Prevalence estimates without adjusting for non-response could lead to overestimation of the prevalence, because individuals with typical FA complaints may have been more likely to participate, which investigation of reported symptomatology during screening in our study did indeed suggest. Soller *et al*. previously evaluated this phenomenon using a Canadian population-based FA survey.[19] They performed multiple imputation on non-participants and found FA prevalence estimates that were lower than the prevalence estimates without imputation. As a result, they recommend multiple imputation to correct for non-response bias in FA prevalence estimates. Application of multiple imputation in this study allowed us to secure unbiased results regarding prevalence of probable FA in Europe.

Results from DBPCFC in our study indicate that around 73% of subjects with probable FA are likely to have a true clinically confirmed FA to common causative foods, which would suggest a population prevalence of 0.2% to 4.1% for confirmed FA. The similarity of this prevalence of challenge-confirmed FA compared to that reported in the systematic review by Nwaru *et al*, 0.1% to 3.2%, support the accuracy of this estimate.[4] However, we advise caution on interpretation of this derived prevalence estimate for challenge-confirmed FA, because this result was based on a limited number of challenge tests.

Prevalence of the various FA estimates was highest in Switzerland and Spain and lowest in Eastern Europe and Greece. An important explanation for differences between countries regarding the prevalence of FA and causative foods is likely to be found in pollen sensitisation patterns. Birch pollen is known to be the most common tree pollen in Northern, Central and Eastern Europe.[20] It is notorious for causing pollen-related FA based on cross-reactivity of IgE to the major birch pollen allergen Bet v 1 with homologous food allergens present in tree nuts (such as hazelnut), *Rosaceae* fruits (such as apple and peach) and *Apiaceae* vegetables (such as carrot and celery).[21,22] In our data, we found that subjects with probable FA were most often co-sensitised to birch pollen in Switzerland (94.7%), the Netherlands (88.6%) and Poland (72.0%) and that hazelnut, apple and peach were indeed the most common causative foods in these countries. Peach was also among the top 3 causative foods in the Mediterranean countries Spain and Greece. In these Mediterranean regions, the high level of sensitisation to lipid transfer proteins (LTPs) is considered the most important cause of FA to LTP-related fruits like peach.[21] In our data, sensitisation to Pru p 3 in subjects with probable FA to peach was indeed most common in Greece (100%) and Spain (44.4%) compared to 0% in Switzerland and the Netherlands. Outside birch pollen territory, cross-reaction with profilins in grasses and ragweed may also play an important role in pollen-related FA. In our data, this could explain the high prevalence of probable FA to melon and wheat in Spain, where grass pollen is abundant.[23] In Switzerland, probable FA to celery and carrot was markedly more

common than in other countries. This could partly be explained by cross-reactivity to mugwort, which is common in Switzerland in addition to birch, and perhaps partly by frequent exposure.

Exposure may also play a key role in the differences observed in causative foods. Data on consumption retrieved from the European Food Safety Authority (EFSA) Comprehensive European Food Consumption Database[24] were available only for children and for 3 of the countries included in this study, but showed that walnut consumption in Greece was 8 times higher than in the Netherlands and 9 times lower than in Spain. Apple consumption in Greece (23 g/d) was lower than in both Spain and the Netherlands (30 g/d and 43 g/d, respectively). As it appears, the more a food is consumed, the more probable FA is detected. In our data, probable FA to walnut was more common in Spain than in Greece and the Netherlands; probable FA to apple was most frequent in the Netherlands followed by Spain and then Greece. Similar results were documented with regard to seafood consumption, which is known to be highest in Southern and Northern Europe.[25] Our study showed that probable FA to seafood was indeed most often seen in these regions: probable FA to shrimp was 1 of the 3 most common probable FAs in Spain and Iceland and probable FA to fish in these 2 countries was the highest in Europe.

Some limitations of this study must be considered. First, estimation of confirmed FA prevalence was compromised by the large proportion of subjects with probable FA refusing challenge testing. Moreover, the prevalence of probable FA was focused on the 24 priority foods and the prevalence of confirmed FA on 6 selected foods, so that non-priority foods were not taken into account in the prevalence estimate. Although this is the most extensive and most recent European population data available for FA, it is somewhat dated, and prevalence may have changed since data collection. Finally, the method of sampling from the general population in Athens differed from that of other centres, because subjects were approached via random digit dialling to mobile phones rather than via a questionnaire sent to a representative sample drawn from local population registers.[9] This led to a very high response rate in Athens (Figure S1) and may have introduced a form of selection bias in this centre.

The main strength of this study is that it is, to our knowledge, the first and only study designed to investigate FA prevalence across Europe using the same study design, questionnaires, challenge materials, and protocols. This made it possible to compare the prevalence of FA across different centres in Europe. Furthermore, multiple imputation was applied to obtain an estimation of probable FA for the complete sample, thus providing the most accurate estimates possible for the prevalence of probable FA, a clinically relevant definition of FA on which data were previously lacking.

In conclusion, this study shows that FA prevalence shows wide variation across Europe, that the foods responsible for the reactions differ per region, and that prevalence and causative foods are likely related to pollen exposure and possibly consumption. Although probable FA was far less common than previously published self-reported FA, prevalence of probable FA was still detected in 0.3% to 5.6% of the Greek, Icelandic, Dutch, Polish, Spanish and Swiss population in increasing order.

Acknowledgements

We would like to thank all the patients for their participation in the study. We acknowledge the support of the EuroPrevall project by the EU (grant FP6-FOOD-CT-2005-514000).

References

1. Ben-Shoshan M, Harrington DW, Soller L, Fragapane J, Joseph L, St Pierre Y, et al. A population-based study on peanut, tree nut, fish, shellfish, and sesame allergy prevalence in Canada. J Allergy Clin Immunol. 2010;125(6): 1327-35.
2. Kanny G, Moneret-Vautrin DA, Flabbee J, Beaudouin E, Morisset M, Thevenin F. Population study of food allergy in France. J Allergy Clin Immunol. 2001;108(1): 133-40.
3. McGowan EC, Keet CA. Prevalence of self-reported food allergy in the National Health and Nutrition Examination Survey (NHANES) 2007-2010. J Allergy Clin Immunol. 2013;132(5): 1216-9.
4. Nwaru BI, Hickstein L, Panesar SS, Muraro A, Werfel T, Cardona V, et al. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. Allergy. 2014;69(1): 62-75.
5. Hadley C. Food allergies on the rise? Determining the prevalence of food allergies, and how quickly it is increasing, is the first step in tackling the problem. EMBO Rep. 2006;7(11): 1080-3.
6. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. J Allergy Clin Immunol. 2007;120(3): 638-46.
7. Zuberbier T, Edenharter G, Worm M, Ehlers I, Reimann S, Hantke T, et al. Prevalence of adverse reactions to food in Germany - a population study. Allergy. 2004;59(3): 338-45.
8. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy. 2014;69(8): 1008-25.
9. Kummeling I, Mills EN, Clausen M, Dubakiene R, Perez CF, Fernandez-Rivas M, et al. The EuroPrevall surveys on the prevalence of food allergies in children and adults: background and study methodology. Allergy. 2009;64(10): 1493-7.
10. Burney PG, Potts J, Kummeling I, Mills EN, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitisation in European adults. Allergy. 2014;69(3): 365-71.
11. Fernandez-Rivas M, Barreales L, Mackie AR, Fritsche P, Vazquez-Cortes S, Jedrzejczak-Czechowicz M, et al. The EuroPrevall outpatient clinic study on food allergy: background and methodology. Allergy. 2015;70(5): 576-84.
12. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. Eur Respir J. 1994;7(5): 954-60.
13. Azur MJ, Stuart EA, Frangakis C, Leaf PJ. Multiple imputation by chained equations: what is it and how does it work? Int J Methods Psychiatr Res. 2011;20(1): 40-9.
14. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. Stat Med. 2011;30(4): 377-99.
15. Graham JW, Olchowski AE, Gilreath TD. How many imputations are really needed? Some practical clarifications of multiple imputation theory. Prev Sci. 2007;8(3): 206-13.
16. Buuren vS, Groothuis-Oudshoorn K. MICE: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software. 2011;45(3): 1-67.
17. Barendregt JJ, Doi SA, Lee YY, Norman RE, Vos T. Meta-analysis of prevalence. J Epidemiol Community Health. 2013;67(11): 974-8.
18. Rubin DB. Multiple imputation for non-response in surveys. New York: Wiley; 1987.
19. Soller L, Ben-Shoshan M, Harrington DW, Knoll M, Fragapane J, Joseph L, et al. Adjusting for nonresponse bias corrects overestimates of food allergy prevalence. J Allergy Clin Immunol Pract. 2015;3(2): 291-3.
20. D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, et al. Allergenic pollen and pollen allergy in Europe. Allergy. 2007;62(9): 976-90.
21. Hofmann A, Burks AW. Pollen food syndrome: update on the allergens. Curr Allergy Asthma Rep. 2008;8(5): 413-7.

22. Werfel T, Asero R, Ballmer-Weber BK, Beyer K, Enrique E, Knulst AC, et al. Position paper of the EAACI: food allergy due to immunological cross-reactions with common inhalant allergens. Allergy. 2015;70(9): 1079-90.
23. Worm M, Jappe U, Kleine-Tebbe J, Schafer C, Reese I, Saloga J, et al. Food allergies resulting from immunological cross-reactivity with inhalant allergens: Guidelines from the German Society for Allergology and Clinical Immunology (DGAKI), the German Dermatology Society (DDG), the Association of German Allergologists (AeDA) and the Society for Pediatric Allergology and Environmental Medicine (GPA). Allergo J Int. 2014;23(1): 1-16.
24. European Food Safety Authority. The EFSA Comprehensive European Food Consumption Database. Available from: https://www.efsa.europa.eu/en/food-consumption/comprehensive-database. Date accessed: August 22, 2018.
25. Directorate-General for Maritime Affairs and Fisheries (European Commission), EUMOFA. EU consumer habits regarding fishery and aquaculture products. Available from: ps://www.eumofa.eu/documents/20178/84590/EU+consumer+habits_final+report+.pdf/5c6348d-a69c-449e-a606-f5615a3a7e4c. Date accessed: August 22, 2018.

Supplemental files

Weighting procedure for population prevalence estimation of probable FA

As described in the methods section, 'probable food allergy' was defined as having symptoms to a specific priority food and sensitisation to the same food. The outcome probable FA was detected among the cases in phase I (those with self-reported FA), hereafter termed 'original cases'.

However, some probable food allergies were also found in the controls, that is, those without self-reported FA to a priority food in phase I who participated in phase II as controls (Figure 1). Of these 'newly detected' cases, those who had also reported symptoms (to nonpriority foods) during screening were included for prevalence estimation of probable FA. These cases are hereafter termed 'new cases'.

Because of the different sampling fractions for the original cases and the new cases, weighting was necessary to calculate the prevalence of probable FA in the population. The total number of probable FAs to priority foods was calculated by adding the number of probable FAs found in the original cases divided by the sampling fraction in the case arm to the number of probable FAs in the new cases divided by the sampling fraction in the control arm. This total number of probable FAs was then divided by the total number of responders to phase I to obtain the population prevalence of probable FA. The equation was as follows:

$$\frac{\dfrac{\text{Number of probable FA in original cases}}{\text{Sampling fraction cases}} + \dfrac{\text{Number of probable FA in new cases}}{\text{Sampling fraction controls}}}{\text{Number of responders in phase I}}$$

The prevalence of probable FA to each priority food as estimated in this way was calculated per country and pooled over all imputations**.** Because some prevalence estimates were very low, 95%-CIs were calculated after applying a double arcsine transformation. Results were then transformed back to obtain an estimate of the prevalence with 95%-CIs for all probable FA prevalence estimates.

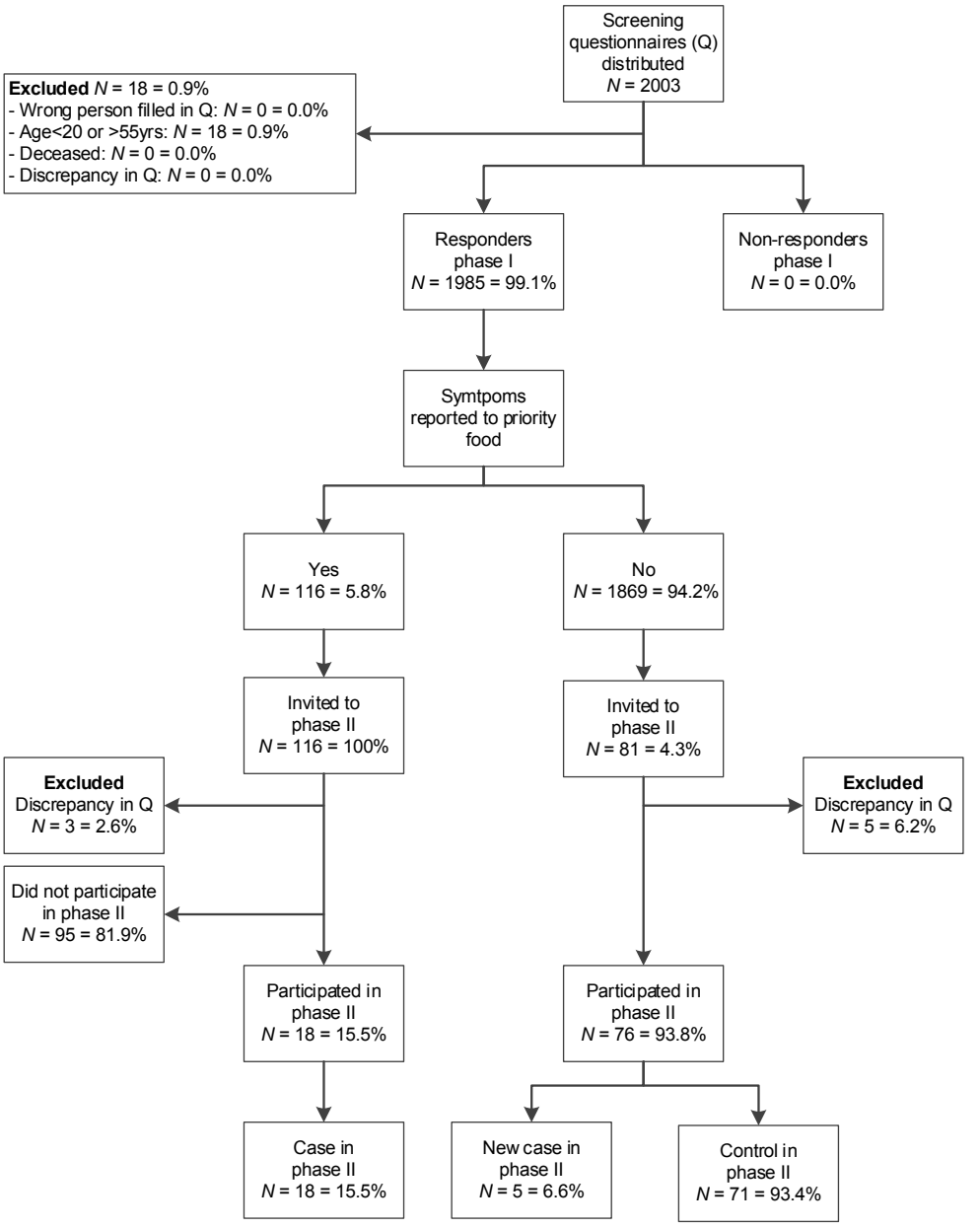

Figure S1. Flowchart Athens

Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but symptoms to only nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I.

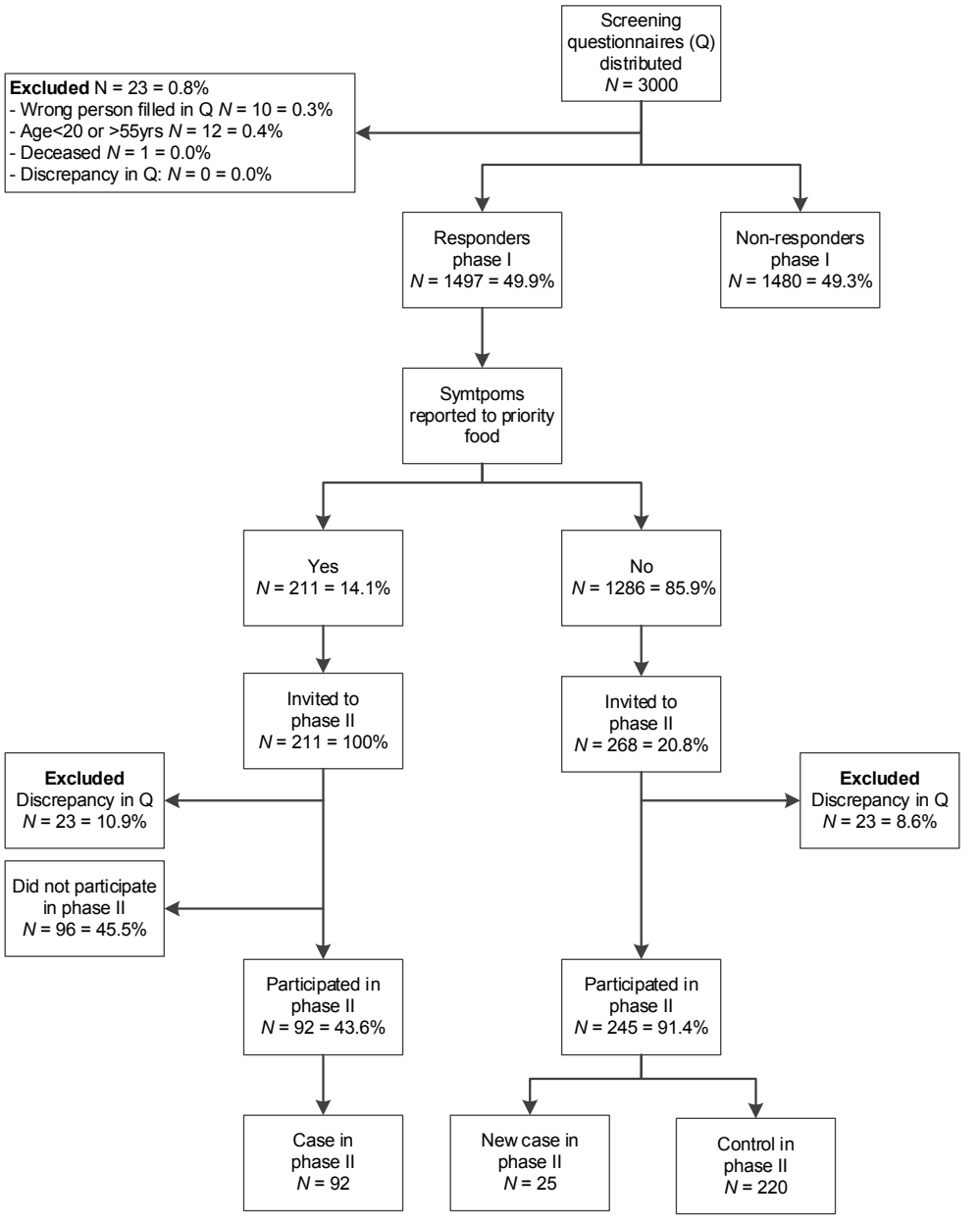

Figure S2. Flowchart Lodz
Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but symptoms to only nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I.

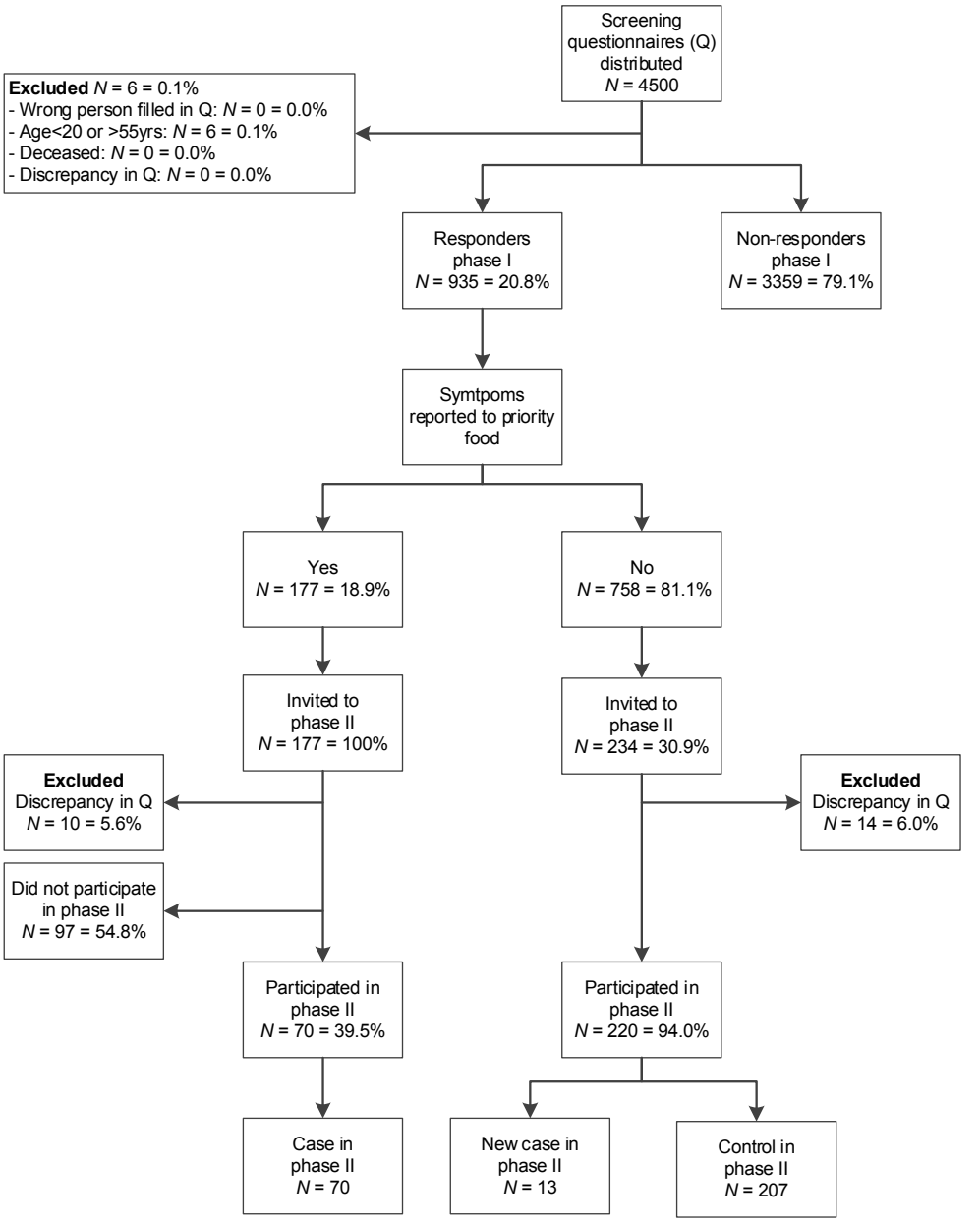

Figure S3. Flowchart Madrid

Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but symptoms to only nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I.

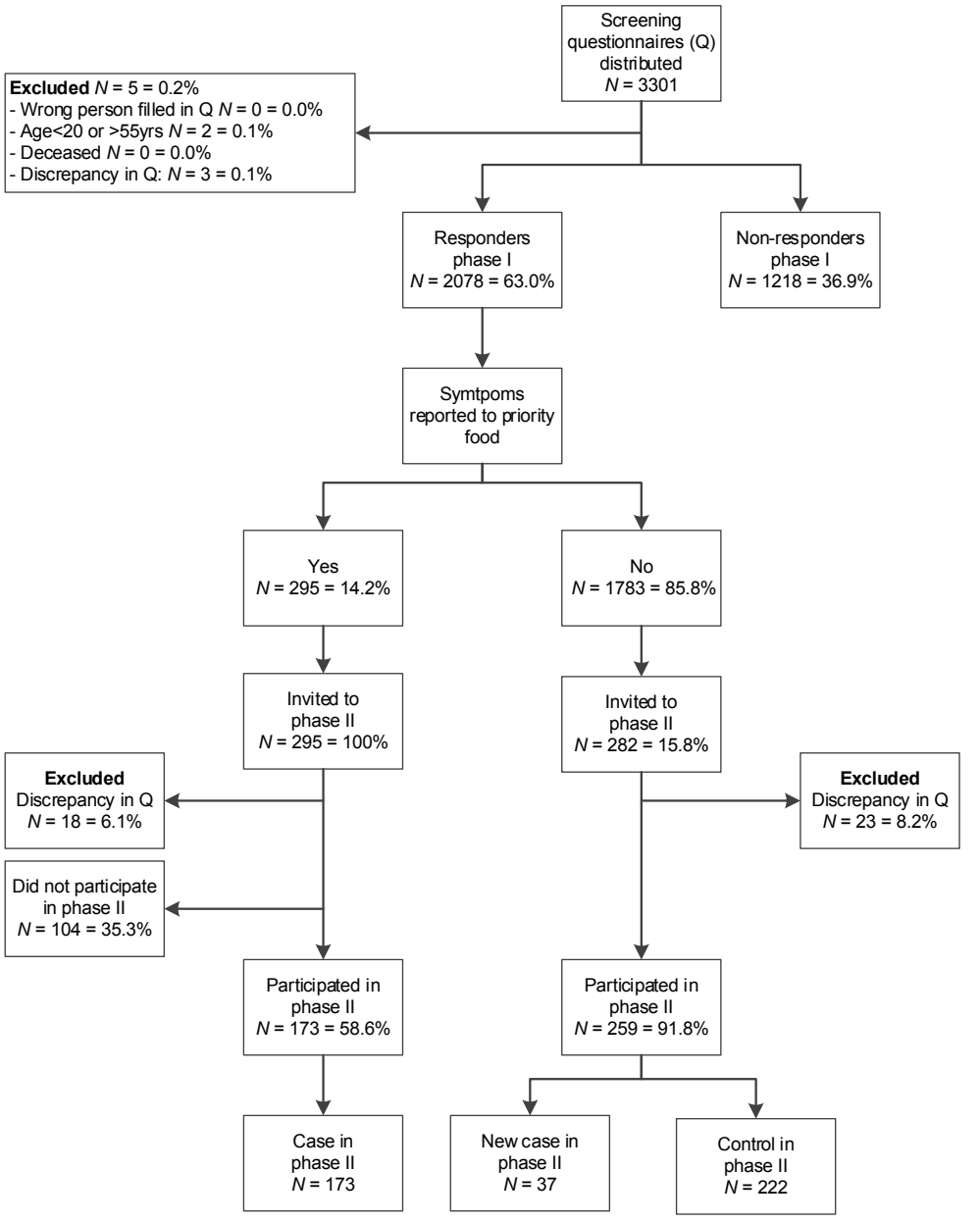

Figure S4. Flowchart Reykjavik
Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but symptoms to only nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I.

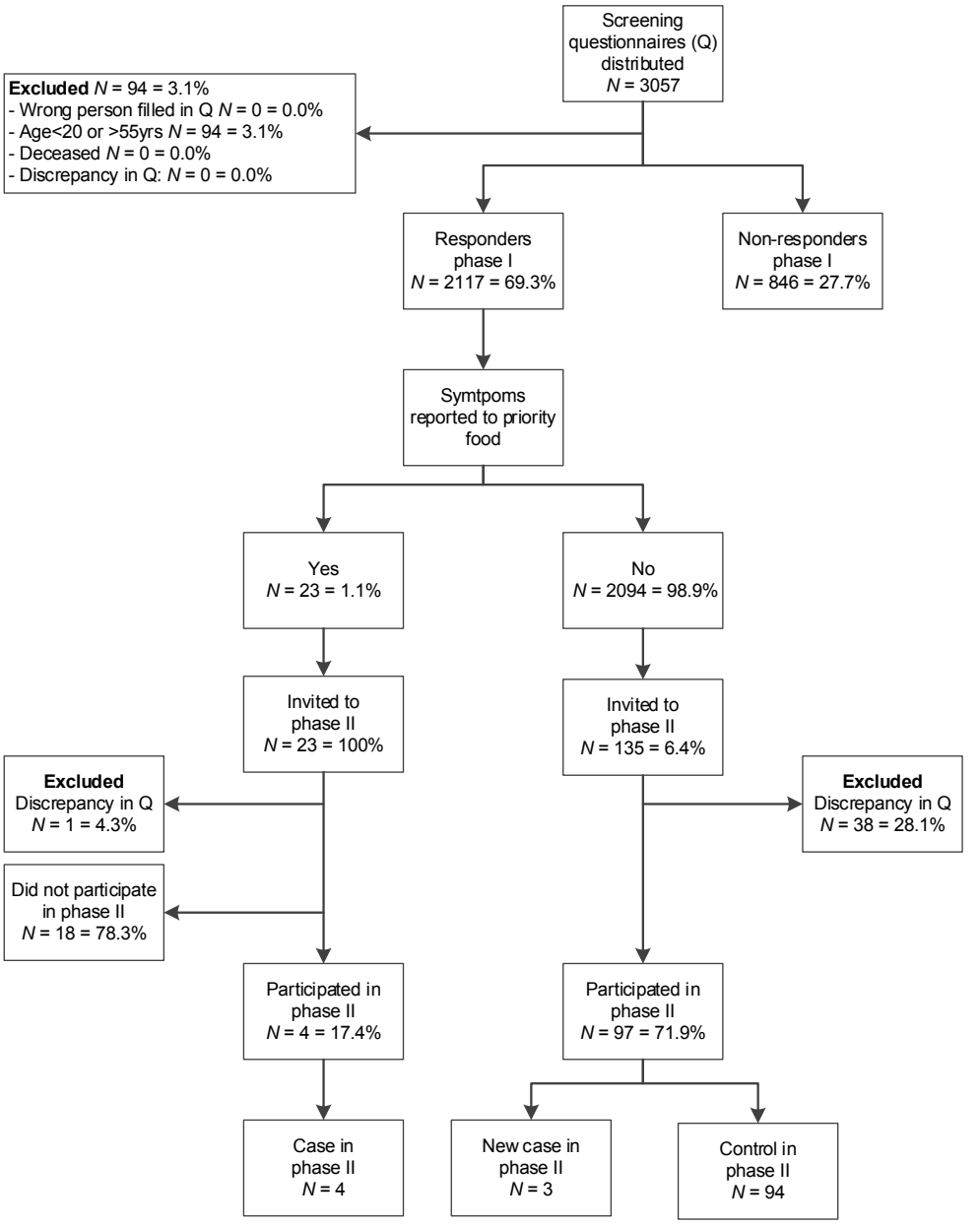

Figure S5. Flowchart Sofia
Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but symptoms to only nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I.

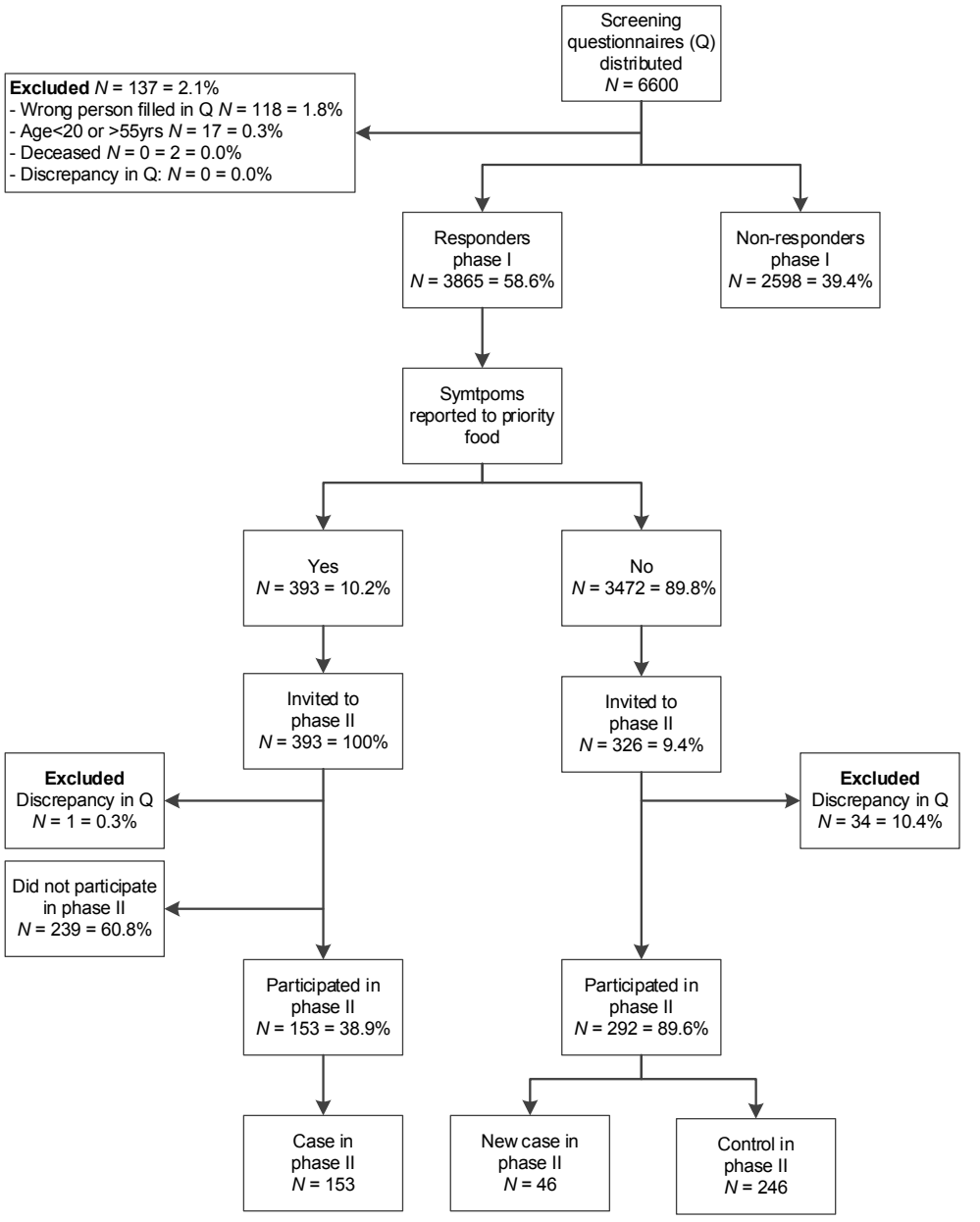

Figure S6. Flowchart Utrecht
Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but symptoms to only nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I.

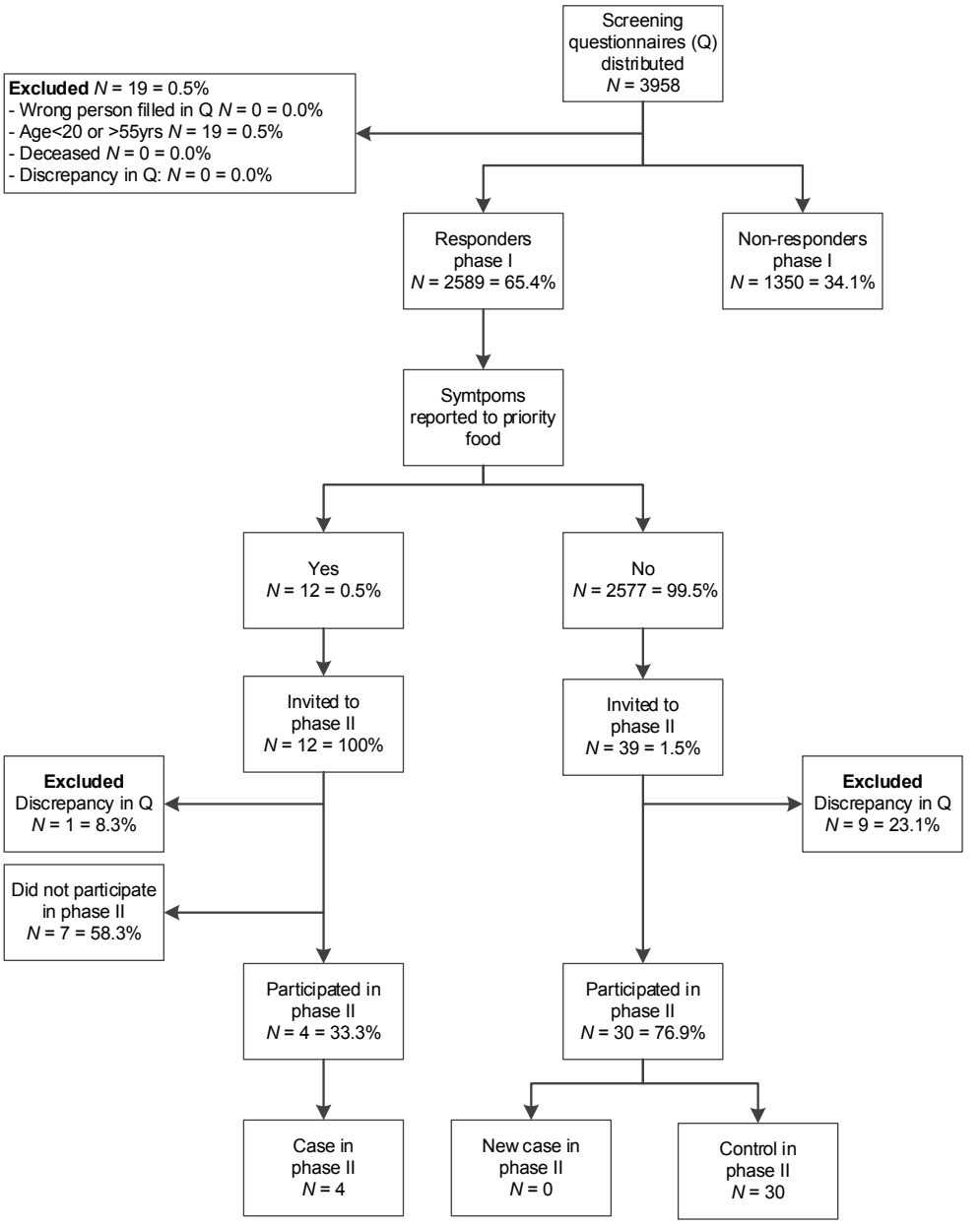

Figure S7. Flowchart Vilnius
Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but symptoms to only nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I.

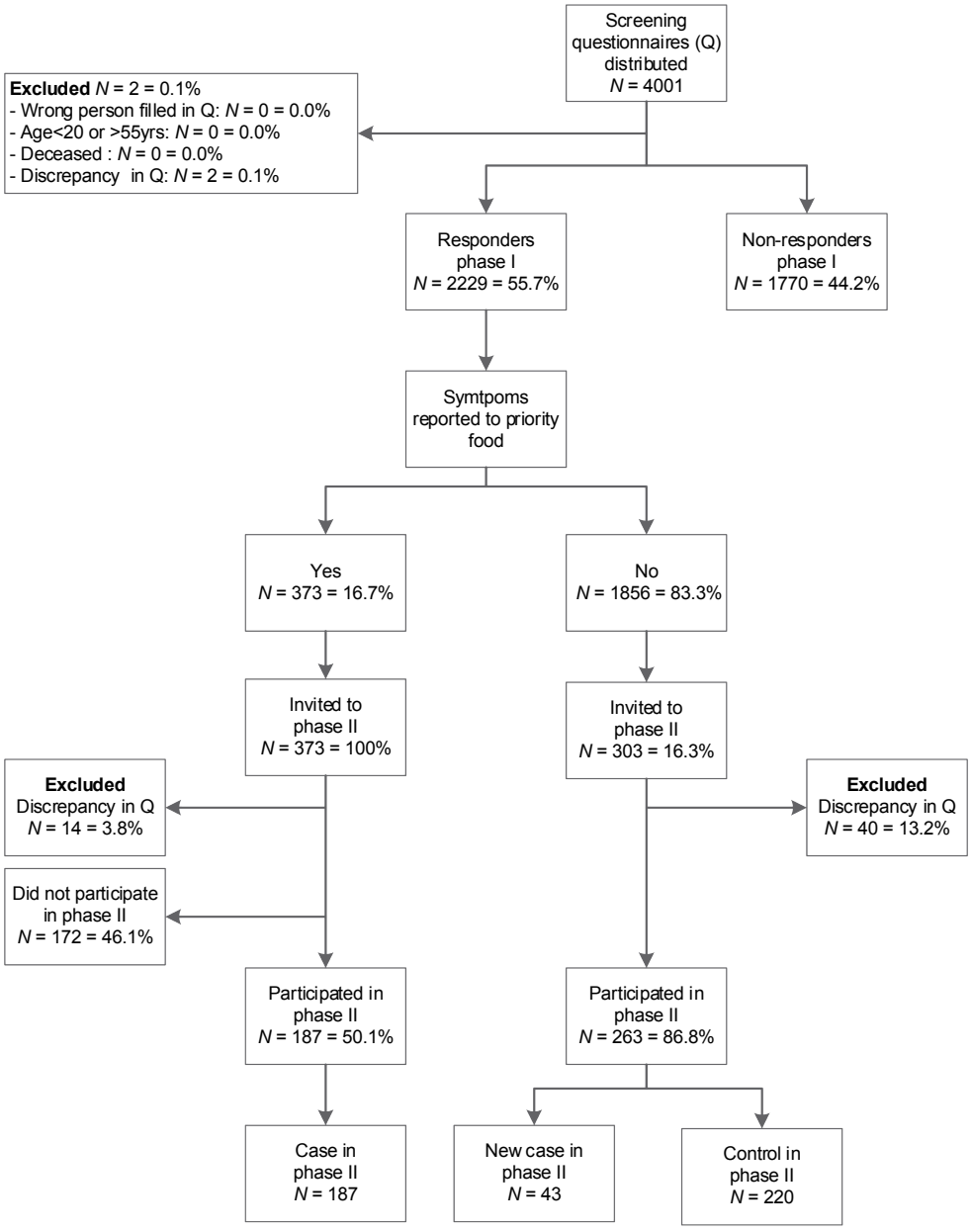

Figure S8. Flowchart Zurich
Case = subject self-reporting symptoms to at least 1 priority food. Control = subject not reporting Case: subject self-reporting symptoms to at least 1 priority food. Control: subject not reporting symptoms to any priority food. New case: subject who reported symptoms to priority foods in phase II, but symptoms to only nonpriority foods in phase I, most likely due to the maximum of 3 foods that could be reported in phase I.

Table S1. Comparison of prevalence estimates of probable FA based on imputed data (I) *versus* complete case analysis (CC)

Prevalence [95%-CI] of probable FA to:	Zurich I	Zurich CC	Madrid I	Madrid CC	Reykjavik I	Reykjavik CC	Lodz I	Lodz CC	Utrecht I	Utrecht CC	Athens I	Athens CC
At least 1 priority food	5.64 [3.94-7.66]	8.39 [5.98-11.21]	3.28 [1.78-5.35]	5.62 [3.29-8.64]	1.36 [0.57-2.54]	1.97 [0.89-3.52]	2.84 [1.51-4.65]	4.70 [2.72-7.26]	2.12 [1.19-3.35]	4.48 [2.76-6.62]	0.31 [0.02-1.65]	1.72 [0.01-6.48]
Hazelnut	2.57 [1.47-4.02]	4.51 [2.77-6.70]	0.76 [0.16-1.96]	1.20 [0.29-2.86]	0.10 [0.00-0.60]	0.16 [0.00-0.82]	1.33 [0.49-2.66]	2.41 [1.06-4.37]	0.93 [0.36-1.81]	2.39 [1.18-4.04]	0.06 [0.19-0.99]	0.34 [0.41-3.41]
Peach	2.02 [1.08-3.30]	3.32 [1.85-5.24]	1.61 [0.63-3.18]	2.67 [1.16-4.92]	0.00 [0.00-0.28]	0.00 [0.00-0.34]	0.45 [0.05-1.35]	0.95 [0.21-2.32]	0.60 [0.17-1.33]	1.53 [0.60-2.90]	0.10 [0.00-1.14]	0.69 [0.16-4.32]
Apple	1.90 [0.99-3.15]	3.37 [1.89-5.31]	0.57 [0.08-1.65]	0.65 [0.07-2.00]	0.05 [0.02-0.48]	0.08 [0.02-0.63]	0.75 [0.17-1.83]	1.58 [0.54-3.24]	0.91 [0.34-1.77]	2.33 [1.14-3.95]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Kiwi	1.34 [0.60-2.42]	1.65 [0.68-3.10]	0.64 [0.11-1.77]	0.92 [0.17-2.44]	0.31 [0.02-0.99]	0.49 [0.06-1.41]	0.30 [0.01-1.09]	0.63 [0.08-1.82]	0.57 [0.15-1.29]	0.98 [0.28-2.12]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Carrot	1.01 [0.39-1.97]	1.66 [0.68-3.11]	0.81 [0.18-2.03]	0.75 [0.10-2.17]	0.38 [0.05-1.12]	0.41 [0.04-1.28]	0.15 [0.00-0.80]	0.32 [0.00-1.27]	0.23 [0.01-0.76]	0.60 [0.10-1.55]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Walnut	0.58 [0.11-1.45]	0.67 [0.13-1.69]	0.71 [0.14-1.88]	1.65 [0.53-3.52]	0.05 [0.02-0.48]	0.08 [0.02-0.63]	0.15 [0.00-0.80]	0.32 [0.00-1.27]	0.10 [0.00-0.51]	0.27 [0.00-0.99]	0.29 [0.02-1.61]	1.72 [0.01-6.48]
Melon	0.46 [0.09-1.17]	0.61 [0.11-1.60]	0.95 [0.25-2.24]	1.75 [0.58-3.66]	0.10 [0.00-0.60]	0.16 [0.00-0.82]	0.00 [0.00-0.36]	0.00 [0.00-0.44]	0.05 [0.01-0.39]	0.13 [0.01-0.72]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Shrimp	0.37 [0.05-1.04]	0.42 [0.04-1.29]	0.82 [0.19-2.04]	1.47 [0.43-3.27]	0.36 [0.04-1.08]	0.57 [0.09-1.54]	0.35 [0.02-1.18]	0.35 [0.01-1.33]	0.36 [0.06-0.97]	0.44 [0.04-1.30]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Peanut	0.37 [0.02-1.17]	0.38 [0.03-1.22]	0.45 [0.05-1.45]	1.10 [0.24-2.71]	0.00 [0.00-0.28]	0.00 [0.00-0.34]	0.35 [0.02-1.18]	0.35 [0.01-1.33]	0.05 [0.01-0.39]	0.13 [0.01-0.72]	0.02 [0.00-0.82]	0.00 [0.00-1.84]
Celery	0.24 [0.01-0.81]	0.48 [0.05-1.38]	0.00 [0.00-0.44]	0.00 [0.00-0.55]	0.33 [0.03-1.03]	0.33 [0.02-1.13]	0.07 [0.02-0.63]	0.16 [0.01-0.94]	0.03 [0.03-0.32]	0.07 [0.03-0.56]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Cow's milk	0.24 [0.00-1.02]	0.00 [0.00-0.33]	0.18 [0.00-0.97]	0.27 [0.00-1.32]	0.00 [0.00-0.28]	0.00 [0.00-0.28]	0.12 [0.01-0.74]	0.16 [0.01-0.94]	0.00 [0.00-0.21]	0.00 [0.00-0.30]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Wheat	0.19 [0.00-0.73]	0.38 [0.03-1.22]	0.37 [0.02-1.31]	0.38 [0.01-1.51]	0.10 [0.00-0.60]	0.16 [0.00-0.82]	0.00 [0.00-0.36]	0.00 [0.00-0.44]	0.05 [0.01-0.39]	0.13 [0.01-0.72]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Lentil	0.16 [0.07-0.93]	0.00 [0.00-0.33]	0.00 [0.00-0.44]	0.00 [0.00-0.55]	0.00 [0.00-0.28]	0.00 [0.00-0.34]	0.00 [0.00-0.36]	0.00 [0.00-0.44]	0.03 [0.03-0.32]	0.07 [0.03-0.56]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Tomato	0.10 [0.00-0.55]	0.19 [0.00-0.87]	0.15 [0.01-0.90]	0.27 [0.00-1.32]	0.15 [0.00-0.71]	0.25 [0.00-0.98]	0.30 [0.01-1.10]	0.63 [0.08-1.82]	0.08 [0.01-0.46]	0.20 [0.00-0.86]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Soybean	0.08 [0.02-0.56]	0.10 [0.02-0.66]	0.00 [0.00-0.44]	0.00 [0.00-0.55]	0.00 [0.00-0.28]	0.00 [0.00-0.34]	0.00 [0.00-0.36]	0.00 [0.00-0.44]	0.03 [0.03-0.32]	0.07 [0.03-0.56]	0.00 [0.00-0.68]	0.00 [0.00-0.68]
Banana	0.05 [0.02-0.42]	0.10 [0.02-0.66]	0.04 [0.00-0.65]	0.00 [0.00-0.55]	0.49 [0.08-1.29]	0.58 [0.09-1.55]	0.00 [0.00-0.36]	0.00 [0.00-0.44]	0.05 [0.01-0.39]	0.13 [0.01-0.72]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Corn	0.05 [0.02-0.42]	0.10 [0.02-0.66]	0.00 [0.00-0.44]	0.00 [0.00-0.55]	0.05 [0.02-0.48]	0.08 [0.02-0.63]	0.00 [0.00-0.36]	0.00 [0.00-0.44]	0.00 [0.00-0.21]	0.00 [0.00-0.30]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Sunflower seed	0.05 [0.02-0.42]	0.10 [0.02-0.66]	0.00 [0.00-0.44]	0.00 [0.00-0.55]	0.00 [0.00-0.28]	0.00 [0.00-0.34]	0.00 [0.00-0.36]	0.00 [0.00-0.44]	0.00 [0.00-0.21]	0.00 [0.00-0.30]	0.07 [0.17-1.05]	0.34 [0.41-3.41]
Sesame seed	0.03 [0.00-0.37]	0.00 [0.00-0.33]	0.00 [0.00-0.44]	0.00 [0.00-0.55]	0.00 [0.00-0.28]	0.00 [0.00-0.34]	0.00 [0.00-0.36]	0.00 [0.00-0.44]	0.00 [0.00-0.21]	0.00 [0.00-0.30]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Fish	0.02 [0.00-0.35]	0.00 [0.00-0.33]	0.44 [0.04-1.44]	0.38 [0.01-1.51]	0.15 [0.00-0.71]	0.25 [0.00-0.98]	0.00 [0.00-0.36]	0.00 [0.00-0.44]	0.08 [0.01-0.46]	0.20 [0.00-0.86]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Buckwheat	0.00 [0.00-0.25]	0.10 [0.02-0.66]	0.00 [0.00-0.44]	0.00 [0.00-0.55]	0.00 [0.00-0.28]	0.00 [0.00-0.34]	0.00 [0.00-0.36]	0.00 [0.00-0.44]	0.00 [0.00-0.21]	0.00 [0.00-0.30]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Hen's egg	0.00 [0.00-0.25]	0.00 [0.00-0.33]	0.06 [0.00-0.70]	0.00 [0.00-0.55]	0.21 [0.00-0.81]	0.33 [0.01-1.13]	0.31 [0.01-1.11]	0.63 [0.08-1.82]	0.00 [0.00-0.21]	0.00 [0.00-0.30]	0.02 [0.00-0.84]	0.00 [0.00-1.84]
Mustard seed	0.00 [0.00-0.25]	0.00 [0.00-0.33]	0.00 [0.00-0.44]	0.00 [0.00-0.55]	0.00 [0.00-0.28]	0.00 [0.00-0.34]	0.03 [0.00-0.52]	0.00 [0.00-0.44]	0.00 [0.00-0.21]	0.00 [0.00-0.30]	0.00 [0.00-0.68]	0.00 [0.00-1.84]
Poppy seed	0.00 [0.00-0.25]	0.00 [0.00-0.33]	0.00 [0.00-0.44]	0.00 [0.00-0.55]	0.00 [0.00-0.28]	0.00 [0.00-0.34]	0.00 [0.00-0.36]	0.00 [0.00-0.44]	0.00 [0.00-0.21]	0.00 [0.00-0.30]	0.00 [0.00-0.68]	0.34 [0.41-3.41]

Chapter 4

Predictors of food sensitisation in children and adults across Europe

J Allergy Clin Immunol Pract. 2020; 8(9):3074-3083

Sarah A. Lyons, André C. Knulst, Peter G.J. Burney, Montserrat Fernández-Rivas, Barbara K. Ballmer-Weber, Laura Barreales, Christian Bieli, Michael Clausen, Ruta Dubakiene, Cristina Fernández-Pérez, Monika Jedrzejczak-Czechowicz, Marek L. Kowalski, Ischa Kummeling, Tihomir B. Mustakov, Harmieke van Os-Medendorp, Nikolaos G. Papadopoulos, Todor A. Popov, James Potts, Paraskevi Xepapadaki, Paco M.J. Welsing, E.N. Clare Mills, Ronald van Ree, Thuy-My Le

Abstract

Background
The geographical variation and increase over time in the prevalence of food sensitisation (FS), suggest environmental influences.

Objective
To investigate how environment, infant diet, and demographic characteristics are associated with FS in children and adults, focusing on early-life exposures.

Methods
Data on childhood and adult environmental exposures (including, among others, sibship size, day care, pets, farm environment, and smoking), infant diet (including breastfeeding and timing of introduction to infant formula and solids), and demographic characteristics were collected from 2196 school-age children and 2185 adults completing an extensive questionnaire and blood sampling in the cross-sectional pan-European EuroPrevall project. Multivariable logistic regression was applied to determine associations between the predictor variables and sensitisation to foods commonly implicated in food allergy (specific IgE≥0.35 kU_A/L). Secondary outcomes were inhalant sensitisation and primary (non-cross-reactive) FS.

Results
Dog ownership in early childhood was inversely associated with childhood FS (OR 0.65 [95%-CI 0.48-0.90]), as was higher gestational age at delivery (OR 0.93 [95%-CI 0.87-0.99] per week increase in age). Lower age and male sex were associated with a higher prevalence of adult FS (OR 0.97 [95%-CI 0.96-0.98] per year increase in age, and 1.39 [95%-CI 1.12-1.71] for male sex). No statistically significant associations were found between other evaluated environmental determinants and childhood or adult FS, nor between infant diet and childhood FS, although early introduction of solids did show a trend toward prevention of FS.

Conclusions
Dog ownership seems to protect against childhood FS, but independent effects of other currently conceived environmental and infant dietary determinants on FS in childhood or adulthood could not be confirmed.

Introduction

Prevalence estimates of IgE sensitisation to foods range from 11% to 29% in 7- to 10-year-old children and from 7% to 24% in adults across various European countries. [1,2] Several studies indicate that the prevalence of food sensitisation (FS) in children and adults is rising.[3-7] The geographical variation in prevalence of FS in children and adults of comparable genetic background, and the proposed increase in FS prevalence over time, suggest an effect of environmental factors.[8]

Although FS does not invariably lead to food allergy (FA), it is a prerequisite, and an objective end point that can feasibly be obtained for multiple foods in a large group of participants. Studies investigating environmental predictors for FS are scarce. Some previous studies report a protective effect of certain environmental determinants on FS in children, such as younger maternal age at delivery,[9] higher number of previous pregnancies,[10] exposure to a farm environment,[10, 11] or having childhood pets (dogs or cats).[11] Determinants related to infant diet also may be relevant. Vitamin D insufficiency and late introduction of solid foods, for example, have been associated with an increased likelihood of FS.[12-14] In adults, a link between use of antacids and increased FS has been proposed.[15] Most studies in adults, however, tend to focus on inhalant sensitisation (IS) or other atopic diseases as outcomes.[16, 17] As part of the European Community Respiratory Health Survey, Svanes *et al*. performed extensive analyses on the effect of a multitude of childhood environmental determinants on adult sensitisation to inhalant allergens, and found a protective effect of increasing family size, bedroom sharing and the presence of a dog in the childhood home.[17] Studies on this scale, which include multiple suggested risk factors and allow for mutual adjustment, are not yet available for the outcome FS or FA. Such studies are key to helping us understand differences in prevalence and time trends between and within populations.[8]

This gap in knowledge led to the current study, in which the primary aim was to investigate how environmental, dietary, and demographic determinants are associated with sensitisation to foods that are commonly consumed across Europe and frequently implicated in FA. The focus was on early-life events and exposures, and the outcome was assessed in both children and adults, using data collected from all across Europe in a standardised manner during the European Union-funded EuroPrevall project. Secondary outcomes included IS and primary (non-cross-reactive) FS, in order to determine whether there are differences between predictors for FS and IS, the latter of which was the focus of previous studies in adult populations.[17]

Methods

Study design, setting and subjects

A full description of the methodology of the EuroPrevall study, a cross-sectional cohort study with a case-control design nested within, is available elsewhere.[18] In the current study, we included randomly sampled children (7-10 years) and adults (20-54 years) from the general population of Athens (Greece), Lodz (Poland), Madrid (Spain), Reykjavik (Iceland), Utrecht (the Netherlands), Zurich (Switzerland), and Vilnius (Lithuania), who completed phase I (a short screening questionnaire) and phase II (a detailed questionnaire and blood sampling for detection of IgE against 24 common food allergens [termed *priority foods*] and 6 common inhalant allergens, as summarised in the 'Supplemental methods on data collection'). All phase I participants who reported symptoms to one or more of the 24 priority foods (cases) and a random sample of phase I participants who reported no symptoms to these foods (controls), were invited for phase II. All data were collected between 2007 and 2009 for children, with a median time interval of 5 months between phase I and II; and between 2006 and 2009 for adults, with a median time interval of 8 months. The local ethical committees of all participating centres gave approval for this study, and informed consent was obtained from all participants.

Data collection

The primary outcome, FS, was defined as positive serology (specific IgE ≥0.35 kU_A/L) to at least one of the 24 selected foods. IS was considered present if a subject was sensitised to at least 1 of the 6 selected inhalant allergens. Primary FS, defined as sensitisation occurring through true plant- or animal-derived food allergens, rather than through pollen inhalant allergens cross-reacting with plant food allergens, was determined using an allergen microarray assay (Figure S1). The overlap between the various outcome variables is depicted in Figure S2.

Information on determinants was extracted from answers to the EuroPrevall phase II questionnaire. In addition to the childhood environmental factors that were investigated for both adults and children, infant dietary factors were taken into account for children, and adult environmental factors for adults. Childhood environmental determinants were investigated for exposure before the age of 2 years for children and before the age of 5 years for adults. To give a complete overview, demographic factors were also assessed. More details on data collection are available in the 'Supplemental methods on data collection'.

Data analysis

As an initial exploration, differences between subjects with and without FS were examined using the chi-square test for categorical variables and the two-sample *t*

test for continuous variables. Univariable logistic regression was performed to assess crude associations between each individual determinant and FS (crude odds ratios [ORs]). Multivariable logistic regression including all determinants was then applied to obtain the independent contribution of the determinants to FS (adjusted ORs). Details on missing data are available from Table S1. To ensure optimal power in the multivariable analysis and potentially reduce bias, multiple imputation was performed using fully conditional specification (20 imputations, 20 iterations), for which all variables in Table S1 were included as covariates. Because it is known that the prevalence of FS is variable between countries[1,2], all analyses were controlled for centre by adding centre as a categorical covariate in the model. To better understand the independent contribution of the various determinants, a stepwise approach to model building was chosen. In model I, multivariable analysis was performed with only demographic factors. In model II, childhood environmental determinants were added. In model III, infant dietary determinants were added for children, and adult environmental determinants for adults.

To observe how the association between the determinants and FS changed on adding comorbidity with overlapping pathophysiological mechanisms, we performed additional analyses. First comorbid atopy (asthma, allergic rhinitis, or atopic dermatitis, or at least 1 of these 3 diseases in first-degree relatives, or parental FA [for the paediatric population]), and then co-existing IgE sensitisation to inhalant allergens, was incorporated into model III. To evaluate whether predictors for FS differ from predictors for IS and primary FS, and enable comparison with earlier studies regarding factors associated with sensitisation, we also fitted model III for these outcomes.

Analyses were conducted with SPSS version 25 (IBM Corporation, Armonk, NY). *P* values less than 0.05 were considered statistically significant.

Results

Of the 2326 children and 2256 adults completing the phase II questionnaire, the 2196 children and 2185 adults with available food serology were evaluated in this study. There were no differences in age or sex between those subjects who did and those who did not complete phase II. In subjects who did complete phase II, subjects with available serology appeared somewhat more likely to report symptoms to (priority) foods and were more likely to be male, than subjects with missing serology results, but no other remarkable differences were observed in demographic characteristics or allergic comorbidities (Table S2).

Respectively 494 children and 441 adults were sensitised to at least one of the priority foods. The median age of the children was 8.9 years in both those with and

those without FS, and respectively 50.8% and 49.6% were males (Table 1). The food-sensitised adults had a median age of 35.3 years and 49.0% were males, whereas the non-sensitised adults had a median age of 39.2 years and 41.1% were males (Table 2). Data on all determinants were complete in 76.5% of the children and 82.2% of the adults. In the rest, missing values on one or more of the determinants were estimated by multiple imputation.

Table 1. Characteristics of children and crude associations with FS

	FS (N=494)	**No FS (N=1702)**	**p**	**Crude OR [95%-CI]**
Demographics				
Age in years; *mean (±SD)*	9.0 (±1.0)	9.0 (±1.0)	0.16	1.09 [0.98-1.21]
Sex				
Male	251 (50.8)	845 (49.6)	0.65	1.05 [0.86-1.28]
Female	243 (49.2)	857 (50.4)		
Level of education parents				
High	206 (41.7)	732 (43.0)	0.60	0.95 [0.77- 1.16]
Low	288 (58.3)	969 (57.0)		
Gestational age in weeks; *mean (±SD)*	39.2 (±2.3)	39.5 (±1.9)	**<0.001**	0.93 [0.89- 0.97]
Birth weight in grams; *mean (±SD)*	3344.3 (±561.3)	3399.0 (±600.5)	0.07	0.98 [0.97-1.00]
Birth length in centimetres; *mean (±SD)*	51.1 (±3.3)	51.6 (±3.4)	**0.01**	0.96 [0.93- 0.99]
Childhood environment				
Maternal age in years; *mean (±SD)*	29.8 (±5.2)	29.7 (±5.5)	0.78	1.00 [0.98-1.02]
Number of siblings				
0	97 (19.7)	302 (17.8)	0.05	*Reference*
1	247 (50.2)	859 (50.6)		0.90 [0.69-1.18]
2	118 (24.0)	368 (21.7)		1.00 [0.73-1.36]
3 or more	30 (6.1)	169 (10.0)		0.55 [0.35-0.86]
Number of older siblings				
0	277 (56.3)	857 (50.5)	**0.02**	*Reference*
1	154 (31.3)	591 (34.8)		0.81 [0.64-1.01]
2	51 (10.4)	175 (10.4)		0.90 [0.64-1.26]
3 or more	10 (2.0)	10 (2.0)		0.41 [0.20 -0.77]
Bedroom sharing other children*	135 (27.3)	493 (29.1)	0.45	0.92 [0.73-1.15]
Bedroom sharing older children*	118 (23.9)	428 (25.2)	0.56	0.93 [0.74-1.18]
Day care attendance*	175 (35.4)	698 (41.1)	**0.02**	0.79 [0.64-0.97]
Farm environment*	2 (0.4)	8 (0.5)	1.00	0.86 [0.13-3.45]
Inner city environment*	212 (42.9)	741 (43.5)	0.81	0.97 [0.80-1.19]
Pet dog*	65 (13.2)	429 (86.8)	**0.01**	0.67 [0.50-0.89]
Pet cat*	79 (16.0)	280 (16.5)	0.80	0.97 [0.73-1.26]
Serious respiratory infection*	133 (26.9)	424 (24.9)	0.37	1.11 [0.88-1.39]
Use of antibiotics*	296 (59.9)	1025 (60.3)	0.88	0.98 [0.80-1.21]
Maternal smoking pregnancy	63 (12.8)	25 3 (14.9)	0.23	0.84 [0.62-1.12]
Maternal smoking since birth	149 (30.2)	528 (31.1)	0.70	0.96 [0.77-1.19]
Paternal smoking since birth	201 (40.7)	710 (41.8)	0.67	0.96 [0.78-1.17]
Reflux medication last 6 months	3 (0.6)	17 (1.0)	0.42	0.61 [0.14-1.81]

Table 1. Characteristics of children and crude associations with food sensitisation (continued)

	FS (N=494)	No FS (N=1702)	p	Crude OR [95%-CI]
Infant diet				
Vitamin D supplementation*	374 (80.4)	1324 (82.3)	0.35	0.88 [0.68-1.15]
Breastfeeding duration				
Never	51 (10.4)	173 (10.3)	0.07	*Reference*
≤4 months	173 (35.3)	521 (31.1)		1.13 [0.79-1.62]
4-6 months	80 (16.3)	237 (14.1)		1.15 [0.77-1.72]
>6 months	186 (38.0)	745 (44.5)		0.85 [0.60-1.21]
Cow's milk infant formula	282 (57.9)	982 (58.2)	0.90	0.99 [0.81-1.21]
Soy milk infant formula	38 (7.8)	110 (6.5)	0.31	1.22 [0.82-1.77]
Hypoallergenic infant formula	126 (25.9)	286 (17.0)	**<0.001**	1.71 [1.34-2.17]
Age start infant formula				
Never	91 (18.7)	430 (25.6)	**0.02**	*Reference*
0-4 months	225 (46.3)	742 (44.2)		1.43 [1.10-1.89]
4-6 months	61 (12.6)	188 (11.2)		1.53 [1.06-2.21]
6-11 months	81 (16.7)	253 (15.1)		1.51 [1.08-2.12]
≥11 months	28 (5.8)	66 (3.9)		2.00 [1.21-3.27}
Age introduction solid foods				
0-4 months	76 (15.7)	342 (20.6)	**0.04**	*Reference*
4-6 months	226 (46.8)	738 (44.4)		1.38 [1.04-1.85]
6-11 months	133 (27.5)	463 (27.8)		1.29 [0.95-1.78]
≥11 months	48 (9.9)	120 (7.2)		1.80 [1.18-2.73]
Comorbid atopy	26 (5.3)	189 (11.3)	**<0.001**	2.28 [1.52-3.55]
Parental food allergy	128 (29.0)	377 (24.8)	0.07	1.24 [0.98-1.57]
Pollen sensitisation†	325 (66.7)	178 (10.6)	**<0.001**	16.89 [13.26-21.62]
Inhalant sensitisation overall‡	368 (75.6)	335 (20.0)	**<0.001**	12.39 [9.78-15.78]
Centre				
Zurich	93 (18.8)	211 (12.4)	**<0.001**	2.71 [1.89-3.89]
Madrid	82 (16.6)	196 (11.5)		2.57 [1.78-3.73]
Athens	40 (8.1)	115 (6.8)		2.14 [1.36-3.33]
Utrecht	102 (20.6)	296 (17.4)		2.12 [1.50-3.00]
Lodz	83 (16.8)	370 (21.7)		1.38 [0.97-1.97]
Vilnius	30 (6.1)	121 (7.1)		1.52 [0.93-2.44]
Reykjavik	64 (13.0)	393 (23.1)		*Reference*

Results presented in N (%) unless otherwise specified. *Before the age of 2 years. †IgE sensitisation to birch, grass, mugwort, parietaria. ‡IgE sensitisation to pollen, cat or house dust mite. **Bold** indicates p<0.05. *FS, food sensitisation; OR, odds ratio; CI, confidence interval.*

Table 2. Characteristics of adults and crude associations with food sensitisation

	FS (N=441)	No FS (N=1744)	p	Crude OR [95%-CI]
Demographics				
Age in years; *mean (±SD)*	36.1 (±9.4)	38.9 (±9.5)	**<0.001**	0.97 [0.96-0.98]
Sex				
Male	216 (49.0)	716 (41.1)	**<0.001**	1.38 [1.12-1.70]
Female	225 (51.0)	1028 (58.9)		
Level of education				
High	285 (64.6)	1151 (66.0)	0.59	0.94 [0.76-1.17]
Low	156 (35.4)	593 (34.0)		
Childhood environment				
Maternal age in years; *mean (±SD)*	28.6 (±5.6)	28.0 (±5.9)	0.07	1.02 [1.00-1.03]
Number of siblings				
0	32 (7.3)	128 (7.4)	**0.01**	*Reference*
1	169 (38.7)	582 (33.5)		1.16 [0.77-1.80]
2	122 (27.9)	428 (24.6)		1.14 [0.74-1.79]
3 or more	114 (26.1)	601 (34.6)		0.76 [0.50-1.19]
Number of older siblings				
0	182 (41.6)	697 (40.1)	0.39	*Reference*
1	141 (32.3)	536 (30.8)		1.01 [0.79-1.29]
2	70 (16.0)	269 (15.5)		1.00 [0.73-1.35]
3 or more	44 (10.1)	237 (13.6)		0.71 [0.49-1.01]
Bedroom sharing other children*	236 (53.5)	1058 (60.7)	**0.01**	0.74 [0.60-0.92]
Bedroom sharing older children*	134 (30.6)	650 (37.4)	**0.01**	0.74 [0.59-0.92]
Day care attendance*	251 (58.8)	906 (53.3)	**0.04**	1.25 [1.01-1.55]
Farm environment*	14 (3.2)	105 (6.0)	**0.02**	0.51 [0.28-0.87]
Inner city environment*	147 (33.3)	535 (30.7)	0.28	1.13 [0.90-1.41]
Pet dog*	165 (37.4)	692 (39.7)	0.38	0.91 [0.73-1.13]
Pet cat*	163 (37.0)	696 (39.9)	0.26	0.88 [0.71-1.09]
Serious respiratory infection*	46 (11.3)	211 (13.2)	0.30	0.84 [0.59-1.16]
Adult environment				
Smoking	224 (50.8)	928 (53.2)	0.36	0.91 [0.74-1.12]
Food-related occupation	109 (24.7)	540 (31.0)	**0.01**	0.73[0.57-0.93]
Indigestion medication currently				
No or <1x/year	386 (87.5)	1513 (86.8)	0.15	*Reference*
Yes, <1x/month	35 (7.9)	110 (6.3)		1.25 [0.83-1.84]
Yes, <1x/week	7 (1.6)	30 (1.7)		0.91 [0.37-1.98]
Yes, ≥ 1x/week	13 (2.9)	91 (5.2)		0.56 [0.30-0.98]
Comorbid atopy	28 (6.3)	371 (21.3)	**<0.001**	3.99 [2.72-6.07]
Pollen sensitisation[†]	340 (80.8)	287 (16.7)	**<0.001**	20.96[16.02-17.70]
Inhalant sensitisation overall[‡]	37 (8.8)	437 (25.4)	**<0.001**	30.47[21.66-44.10]
Centre				
Zurich	147 (33.3)	335 (19.2)	**<0.001**	4.88 [3.37-7.23]
Madrid	63 (14.3)	246 (14.1)		2.85 [1.86-4.41]
Athens	15 (3.4)	52 (3.0)		3.21 [1.62-6.12]
Utrecht	112 (25.4)	364 (20.9)		3.42 [2.34-5.11]
Lodz	65 (14.7)	313 (17.9)		2.31 [1.52-3.55]
Reykjavik	39 (8.8)	434 (24.9)		*Reference*

Results presented in N (%) unless otherwise specified. *Before the age of 5 years. [†]IgE sensitisation to birch, grass, mugwort, parietaria. [‡]IgE sensitisation to pollen, cat or house dust mite. **Bold** indicates p<0.05. *FS, food sensitisation; OR, odds ratio; CI, confidence interval.*

Children: crude associations between demographic, childhood environmental, and infant dietary determinants and FS

Table 1 presents the results of univariable analyses in children. No significant associations between demographic factors, age, sex and parental level of education with FS in childhood were found. Lower gestational age, lower birth weight and shorter length at birth tended to increase the likelihood of FS (Table 1). Comorbid atopy, parental FA, and IS also showed strong positive associations with FS.

Many childhood environmental factors demonstrated significant inverse associations with FS, specifically having more siblings, having older siblings, day care attendance before the age of 2 years, and having a pet dog before the age of 2 years.

Regarding dietary determinants, breastfeeding for longer than 6 months was inversely associated with FS, compared with breastfeeding for less than 6 months. Breastfeeding was not necessarily exclusive. Use of infant formula, which could be either a replacement of or complementary to breastfeeding, was positively associated with FS; especially hypoallergenic infant formula. In subjects who received infant formula, a trend was suggested where the younger the infant formula was introduced, the less likely FS became. A similar trend was observed for solid food introduction.

Independent predictors of food sensitisation in children

In multivariable analyses, gestational age was the only demographic determinant that remained significantly associated with FS in childhood (Table 3). The longer the pregnancy, the lower the chance of FS in the child (OR 0.93 [95%-CI 0.87-0.99] per week of pregnancy duration). Of the childhood environmental determinants, having a pet dog before the age of two years was still significantly inversely associated after multivariable adjustment (OR 0.65 [95%-CI 0.48-0.90]). Of the infant dietary determinants, use of hypoallergenic infant formula maintained a positive association with childhood FS (OR 1.51 [95%-CI 1.06-2.15]).

Adjustment for comorbid atopy did not result in relevant changes to the associations obtained in model III (Table S3). Subsequent correction for co-existing IS resulted in male sex becoming significantly inversely associated with FS (OR 0.64 [95%-CI 0.49-0.84]) and attenuated the associations between having a pet dog before the age of 2 years and use of hypoallergenic infant formula and FS in childhood, suggesting that reverse causality explains part of the observed associations (Table S3). The association between pet dog and FS remained significant.

Adults: crude associations between demographic, childhood and adult environmental determinants, and FS

Table 2 presents the results of univariable analyses in adults. Of the demographic factors, younger age and male sex were significantly positively associated with FS, as were comorbid atopy and IS.

Analyses of childhood environmental determinants in adults showed a significant inverse association between having more siblings, sharing a bedroom with other or older children before the age of 5 years, and growing up on a farm before the age of 5 years and FS in adulthood (Table 2). In contrast to the findings in children, day care attendance before the age of 5 years was shown to significantly increase the likelihood of adult FS. Of the adult environmental determinants, ever having had a food-related occupation was associated with a lower risk of FS.

Independent predictors of food sensitisation in adults

Only the demographic factors age and sex remained significantly associated with adult FS after multivariable adjustment (Table 3), yielding an OR of 0.97 [95%-CI 0.96-0.98] per year increase in age, and OR of 1.39 [95%-CI 1.12-1.71] for male sex. Multivariable analyses revealed no significant associations between childhood or adult environmental determinants and FS in adults. Addition of comorbid atopy and subsequently IS to the model did not notably change any of the observed associations (Table S3).

Table 3. Independent predictors of food sensitisation

	Children		**Adults**	
	Adjusted OR [95%-CI]	**p**	**Adjusted OR [95%-CI]**	**p**
Demographics				
Age per year	1.01 [0.91-1.13]	0.85	**0.97 [0.96-0.98]**	**<0.001**
Male sex	1.01 [0.82-1.25]	0.92	**1.39 [1.12-1.73]**	**<0.001**
High level of education*	1.02 [0.80-1.29]	0.88	0.86 [0.67-1.09]	0.21
Gestational age per week	**0.93 [0.87-0.99]**	**0.01**	NA	NA
Birth weight per 100 grams	1.00 [1.00-1.00]	0.97	NA	NA
Birth length per centimetre	1.01 [0.97-1.06]	0.65	NA	NA
Childhood environment				
Maternal age per year	0.99 [0.97-1.01]	0.33	1.01 [0.99-1.03]	0.45
Number of siblings				
1	0.86 [0.63-1.18]	0.28	1.22 [0.75-1.97]	0.74
2	1.09 [0.73-1.64]		1.34 [0.78-2.29]	
3 or more	0.76 [0.40-1.44]		1.35 [0.75-2.42]	
Number of older siblings				
1	0.85 [0.63-1.15]	0.62	1.17 [0.85-1.62]	0.74
2	1.02 [0.63-1.18]		1.21 [0.78-1.88]	
3 or more	0.77 [0.31-1.95]		1.22 [0.69-2.14]	

Table 3. Independent predictors of food sensitisation (continued)

	Children		**Adults**	
	Adjusted OR [95%-CI]	**p**	**Adjusted OR [95%-CI]**	**p**
Bedroom sharing other children	0.77 [0.53-1.13]†	0.18	0.84 [0.62-1.14]‡	0.26
Bedroom sharing older children	1.19 [0.79-1.80]†	0.40	0.75 [0.52-1.06]‡	0.11
Day care attendance	0.82 [0.64-1.05]†	0.12	1.02 [0.80-1.30]‡	0.89
Farm environment	1.06 [0.21-5.36]†	0.95	0.73 [0.40-1.35]‡	0.32
Inner city environment	0.94 [0.76-1.17]†	0.60	1.01 [0.79-1.29]‡	0.93
Pet dog	**0.65[0.48-0.90]**†	**0.01**	0.94 [0.70-1.27]‡	0.61
Pet cat	1.04 [0.77-1.40]†	0.79	0.87 [0.68-1.10]‡	0.24
Serious respiratory infection	1.11 [0.86-1.45]†	0.42	0.88 [0.62-1.25]‡	0.47
Use of antibiotics	1.14 [0.89-1.45]†	0.29	NA	NA
Maternal smoking pregnancy	0.74 [0.51-1.08]	0.12	NA	NA
Maternal smoking since birth	1.12 [0.84-1.48]	0.45	NA	NA
Paternal smoking since birth	0.97 [0.77-1.22]	0.78	NA	NA
Reflux medication last 6 months	0.61 [0.17-2.19]	0.45	NA	NA
Infant diet				
Vitamin D supplementation	1.05 [0.75-1.47] †	0.77	NA	NA
Breastfeeding duration				
≤4 months	1.20 [0.82-1.75]	0.29	NA	NA
4-6 months	1.20 [0.75-1.94]		NA	NA
>6 months	0.91 [0.57-1.46]		NA	NA
Cow's milk infant formula	0.85 [0.58-1.24]	0.39	NA	NA
Soy milk infant formula	1.35 [0.89-2.05]	0.16	NA	NA
Hypoallergenic infant formula	**1.51 [1.06-2.15]**	**0.02**	NA	NA
Age start infant formula				
0-4 months	1.02 [0.60-1.72]	0.29	NA	NA
4-6 months	1.04 [0.60-1.79]		NA	NA
6-11 months	1.10 [0.66-1.82]		NA	NA
≥11 months	1.79 [0.94-3.40]		NA	NA
Age introduction solid foods				
4-6 months	1.37 [1.00-1.86]	0.33	NA	NA
6-11 months	1.22 [0.86-1.71]		NA	NA
≥11 months	1.45 [0.93-2.27]		NA	NA
Adult environment				
Smoking	NA	NA	0.99 [0.79-1.24]	0.94
Food-related occupation	NA	NA	0.83 [0.64-1.07]	0.15
Indigestion medication currently				
<1x/month	NA	NA	1.30 [0.85-1.97]	0.59
<1x/week	NA	NA	1.10 [0.46-2.60]	
≥ 1x/week	NA	NA	0.84 [0.46-1.54]	

Analyses were adjusted for centre. *For children: high level of education parents. †Before the age of 2 years. ‡Before the age of 5 years. Reference categories: "number of (older) siblings" = 0; "indigestion medication" = no or <1x/year; "breastfeeding duration" = never; "age start infant formula" = never; "age introduction solid food = 0-4 months. **Bold**: p<0.05. *HDM, house dust mite; OR, odds ratio; CI, confidence interval; NA, not available.*

Secondary outcomes: independent predictors of sensitisation to inhalant allergens and primary food allergens

Table S4 facilitates comparison of predictors for FS, as discussed above, with predictors of the secondary outcomes, IS and primary FS. In children, the results pertaining to IS were generally similar to those related to FS. Noticeable exceptions were that bedroom sharing with other children before the age of 2 years and growing up in a farm environment showed stronger inverse associations with IS, the former of which reached statistical significance. Also, although sex was not associated with FS, male sex was significantly positively associated with IS in children. Regarding primary FS in children, no particular differences in predictors were found compared with overall FS.

In adults, it was noteworthy that where there were no significant associations between the childhood environmental determinants and FS in adulthood, there was a significant inverse association between growing up in a farm environment or with a pet cat before the age of 5 years and IS. Furthermore, increasing maternal age was significantly associated with higher risk of IS. Adjustment for comorbid atopy attenuated the protective effect of cat ownership, but not that of farm or maternal age, which suggests some reverse causality in the association between cat ownership and IS.

Regarding the outcome primary FS, bedroom sharing with older children before the age of 5 years was found to be inversely associated, and smoking in adulthood significantly increased the likelihood of primary FS in adults.

Discussion

Summary of findings

Until now, no study existed in which the main postulated environmental and infant dietary risk factors for FA were evaluated collectively in multivariable analysis, especially not all over Europe for both children and adults. The data from the EuroPrevall project made this possible. In our study, having a pet dog before the age of 2 years was the only statistically significantly predictive early-life exposure, and it was found to be strongly associated with a decreased risk of FS in childhood. Higher gestational age at birth was also significantly inversely associated with childhood FS. No childhood or adult environmental determinants were significantly associated with FS in adulthood, but demographic characteristics lower age and male sex did make adult FS more likely.

Early-life exposures: environmental determinants

The finding that having a pet dog in early childhood was inversely associated with FS consolidates existing evidence that dog ownership protects against childhood

FA.[19-21] The protective effect is thought to be the result of exposure to diverse environmental microbiota.[21] Similarly to the adjusted OR of 0.65 (95%-CI 0.48-0.90) in the current study, Koplin *et al*. reported an adjusted OR of 0.6 (95%-CI 0.3-0.8) for the association between dog ownership and challenge-proven egg, peanut and sesame allergy in Australian infants participating in the HealthNuts study;[22] and Von Hertzen *et al*. found an adjusted OR of 0.57 (95%-CI 0.35-0.95) for the association between having a dog before the age of 1 year and food and inhalant sensitisation in Finnish schoolchildren aged 7 to 16 years.[11] More recent findings from Europe by Marrs *et al*. demonstrate an even stronger relationship, with an adjusted OR of 0.1 (95%-CI 0.01-0.71) between dog ownership before 3 months and challenge-confirmed FA to cow's milk, egg white, cod, peanut, sesame and wheat in infants aged 1 to 3 years from the United Kingdom and Wales.[21] Perhaps the association is stronger depending on how young the subject is at exposure, or depending on how old the subject is when the outcome is measured. Our finding that having a pet dog in childhood was not as strongly inversely associated with FS in adulthood as with FS in childhood, might be explained by the fact that the exposure to dog before the age of 5 years instead of 2 years was examined in adults. Or maybe the protective effect does not last until adulthood. Alternatively, one should consider the fact that the adults stemmed from an environmentally different childhood era than the children in this study, which could mean that predictors for the current adults differ from those for the current children when reaching adulthood.

Previous literature suggests that environmental factors other than having a pet dog in childhood also influence the likelihood of FA.[23-26] Two environmental determinants that are relatively consistently suggested to protect against FA in children are an increasing number of siblings, especially older siblings,[23-26] and rural or farm lifestyle.[23, 27, 28] In our study, these determinants also exhibited an inverse association with FS in univariable analysis, both for children and adults. However, the suggested protective effects were attenuated and lost statistical significance in multivariable analysis. Although the current study was not specifically powered to detect associations (of minimal relevant size) for all predictors studied, nor was it designed to asses causal associations, our study is unique in that it included both childhood environmental exposures and infant dietary determinants in multivariable analysis for prediction of childhood FS, which may influence the associations found in comparison with earlier studies where a more limited set of predictive factors was evaluated. Furthermore, as the outcome FA is often (partly) defined by a measure of subjective interpretation, subjects self-reporting symptoms may be more likely to report risk factors associated with FA than subjects without symptoms, thus possibly inflating associations. Our objective outcome, FS, may have mitigated these associations.

Nonetheless, several studies also report a significant protective effect of early-life farm exposure on IgE sensitisation, in both children[11, 29] and adults [17, 30]. All these studies, however, focused on IS. In our study, early-life farm exposure, but also bedroom sharing in childhood for example, was more strongly inversely associated with IS than with FS in both children and adults, to the point of reaching statistical significance. Even larger differences were observed when comparing IS to primary FS. It is possible that predictors for FS and IS differ (in effect size), despite shared pathophysiological mechanisms.

Early-life exposures: infant diet

Regarding infant diet, none of the dietary determinants evaluated in our study significantly predicted FS in childhood, except for use of hypoallergenic infant formula. Findings from models with further adjustment for comorbid atopy and IS, where the association between the use of hypoallergenic infant formula and FS lost statistical significance, suggest that reverse causality may play a role here. In other words, food-sensitised infants may be more likely to receive hypoallergenic infant formula rather than the other way around.

Literature is inconsistent on whether breastfeeding is protective against allergy, neutral or allergy-promoting.[31] In accordance with our findings, most systematic reviews of literature conclude that there appears to be no significant association between breastfeeding ever *versus* never, or breastfeeding duration, and FA.[23, 32, 33] Interestingly, Hong *et al*. found that breastfeeding significantly increased or decreased the odds of FS in childhood depending on genotype, implying that individual genes may decide whether breastfeeding promotes or protects against FS.[31]

No significant trend was found for timing of introduction of infant formula or solid foods and likelihood of FS in childhood in multivariable analyses, but effect direction did suggest that earlier introduction of solid foods may protect against FS. Introduction before 4 months would appear to be associated with the least likely FS, as was also concluded in a recent systematic review and meta-analyses by Burgess *et al*.[34]

Demographic characteristics

Demographic factors were taken into account for complete adjustment, and as expected, some were relevant predictors of FS in our data. Interestingly, higher gestational age at delivery was found to be inversely related to FS in childhood. Available literature on the relationship between gestational age and the outcome FS is scarce, and previously showed no significant relationship[35]. For the outcome FA, some results are contradictory in that they reveal a positive association between preterm birth and FA,[19, 36] whereas others again report no significant

relationship.[37, 38] Several studies suggest that immune system homeostasis, gut barrier function, and diversity in gut microbiota are essential for normal tolerance development.[39, 40] Because these features are underdeveloped in preterm infants, this may make FS more likely. Another possibility is that there is confounding through caesarean section, on which we had no data. Systematic reviews have concluded that caesarean section, which is more common in preterm births, is positively associated with both FS and FA, mostly based on the theory that babies born through caesarean section are deprived of first colonisation of the gut with maternal vaginal bacteria.[39, 41]

In adults, FS significantly decreased with age in our study. Our data on primary FS in children suggest the same effect direction for children, but probably did not reach statistical significance due to the small age range in included children (7-10 years). Similar trends were found in cross-sectional studies in American, Italian and German children and adults.[42-44] These trends may be the result of increasing prevalence of FS over time, as suggested by some studies,[3, 6, 7] which would lead to a higher prevalence of sensitisation in the younger age groups.

Male sex was positively associated with FS in adults. The latter finding appears to contradict the predominating thought that women are at a higher risk of FA than men.[23, 26, 32, 45] However, with regard to FS, available studies also found that adult males are more likely to be food sensitised than adult females.[42, 44] Because many studies investigate the outcome FA rather than FS, outcome measures are mainly based on patient history. Because women are more likely than men to report symptoms to foods,[44, 46, 47] this may explain the discrepancy.

Strengths and limitations

Overall, this study is unique in that data on determinants were analysed for the outcomes FS, IS and primary FS, in both children and adults, after assessing IgE sensitisation to 24 foods. A limitation is the retrospective data collection on childhood determinants, which means that some recall bias is likely, especially in adults. Also, causal inference was limited, because we had no information on when sensitisation developed in relation to the exposure variables. We did attempt to take the possibility of reverse causality into account, assessing the change in associations after adjusting for comorbid atopy. The standardised approach of this multicentre study all across Europe is a major strength, allowing valid comparisons. The broad inclusion from the general population and the use of multiple imputation for sporadically missing data on determinants yielded a large study population in which we could evaluate most currently conceived environmental, infant dietary and demographic risk factors.

Conclusions
Our findings consolidate existing evidence that dog ownership in early childhood protects against FS in later childhood. Other postulated environmental and infant dietary risk factors for FA appear to have (more) limited impact on childhood or adult FS after mutual adjustment, though preventative tendencies were observed for certain early-life exposures, including early introduction of solid foods. Demographic factors also appear relevant, in that gestational age affects the likelihood of childhood FS, and age and sex the likelihood of adult FS.

Acknowledgements

We would like to thank all the patients for their participation in the study. We acknowledge the support of the EuroPrevall project by the EU (grant FP6-FOOD-CT-2005-514000).

References

1. Lyons SA, Clausen M, Knulst AC, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, et al. Prevalence of food sensitization and food allergy in children across Europe. J Allergy Clin Immunol Pract. 2020; 8(8):2736-2746.e9.
2. Burney PG, Potts J, Kummeling I, Mills EN, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitisation in European adults. Allergy. 2014;69(3):365-71.
3. Ezendam J, Klis vdFR, Loveren vH. Time trends in prevalence of sensitisation to milk, egg and peanut in the Netherlands. Contract No.: 340350003. Bilthoven, The Netherlands: National Institue for Public Health and the Environment of The Netherlands; 2009.
4. Ronmark E, Bjerg A, Perzanowski M, Platts-Mills T, Lundback B. Major increase in allergic sensitisation in schoolchildren from 1996 to 2006 in northern Sweden. J Allergy Clin Immunol. 2009;124(2):357-63, 63 e1-15.
5. Koet LBM, Brand PLP. Increase in atopic sensitisation rate among Dutch children with symptoms of allergic disease between 1994 and 2014. Pediatr Allergy Immunol. 2018;29(1):78-83.
6. Hu Y, Chen J, Li H. Comparison of food allergy prevalence among Chinese infants in Chongqing, 2009 versus 1999. Pediatr Int. 2010;52(5):820-4.
7. Mullins RJ, Dear KB, Tang ML. Characteristics of childhood peanut allergy in the Australian Capital Territory, 1995 to 2007. J Allergy Clin Immunol. 2009;123(3):689-93.
8. Papadopoulos NG, Agache I, Bavbek S, Bilo BM, Braido F, Cardona V, et al. Research needs in allergy: an EAACI position paper, in collaboration with EFA. Clin Transl Allergy. 2012;2(1):21.
9. Dioun AF, Harris SK, Hibberd PL. Is maternal age at delivery related to childhood food allergy? Pediatr Allergy Immunol. 2003;14(4):307-11.
10. Ege MJ, Herzum I, Buchele G, Krauss-Etschmann S, Lauener RP, Roponen M, et al. Prenatal exposure to a farm environment modifies atopic sensitisation at birth. J Allergy Clin Immunol. 2008;122(2):407-12, 12 e1-4.
11. von Hertzen L, Makela MJ, Petays T, Jousilahti P, Kosunen TU, Laatikainen T, et al. Growing disparities in atopy between the Finns and the Russians: a comparison of 2 generations. J Allergy Clin Immunol. 2006;117(1):151-7.
12. Nwaru BI, Ahonen S, Kaila M, Erkkola M, Haapala AM, Kronberg-Kippila C, et al. Maternal diet during pregnancy and allergic sensitisation in the offspring by 5 yrs of age: a prospective cohort study. Pediatr Allergy Immunol. 2010;21(1 Pt 1):29-37.
13. Nwaru BI, Erkkola M, Ahonen S, Kaila M, Haapala AM, Kronberg-Kippila C, et al. Age at the introduction of solid foods during the first year and allergic sensitisation at age 5 years. Pediatrics. 2010;125(1):50-9.
14. Zutavern A, Brockow I, Schaaf B, von Berg A, Diez U, Borte M, et al. Timing of solid food introduction in relation to eczema, asthma, allergic rhinitis, and food and inhalant sensitisation at the age of 6 years: results from the prospective birth cohort study LISA. Pediatrics. 2008;121(1):e44-52.
15. Untersmayr E, Bakos N, Scholl I, Kundi M, Roth-Walter F, Szalai K, et al. Anti-ulcer drugs promote IgE formation toward dietary antigens in adult patients. FASEB J. 2005;19(6):656-8.
16. Matheson MC, Dharmage SC, Abramson MJ, Walters EH, Sunyer J, de Marco R, et al. Early-life risk factors and incidence of rhinitis: results from the European Community Respiratory Health Study--an international population-based cohort study. J Allergy Clin Immunol. 2011;128(4):816-23 e5.
17. Svanes C, Jarvis D, Chinn S, Burney P. Childhood environment and adult atopy: results from the European Community Respiratory Health Survey. J Allergy Clin Immunol. 1999;103(3 Pt 1):415-20.
18. Kummeling I, Mills EN, Clausen M, Dubakiene R, Perez CF, Fernandez-Rivas M, et al. The EuroPrevall surveys on the prevalence of food allergies in children and adults: background and study methodology. Allergy. 2009;64(10):1493-7.

19. Koplin JJ, Dharmage SC, Ponsonby AL, Tang ML, Lowe AJ, Gurrin LC, et al. Environmental and demographic risk factors for egg allergy in a population-based study of infants. Allergy. 2012;67(11):1415-22.
20. Lodge CJ, Allen KJ, Lowe AJ, Hill DJ, Hosking CS, Abramson MJ, et al. Perinatal cat and dog exposure and the risk of asthma and allergy in the urban environment: a systematic review of longitudinal studies. Clin Dev Immunol. 2012;2012:176484.
21. Marrs T, Logan K, Craven J, Radulovic S, Irwin McLean WHA, Lack G, et al. Dog ownership at three months of age is associated with protection against food allergy. Allergy. 2019;74(11):2212-2219.
22. Koplin JJ, Martin PE, Tang ML, Gurrin LC, Lowe AJ, Osborne NJ, et al. Do factors known to alter infant microbial exposures alter the risk of food allergy and eczema in a population-based infant study? J Allergy Clin Immunol. 2012;129(2).
23. Ben-Shoshan M, Turnbull E, Clarke A. Food allergy: temporal trends and determinants. Curr Allergy Asthma Rep. 2012;12(4):346-72.
24. Marrs T, Bruce KD, Logan K, Rivett DW, Perkin MR, Lack G, et al. Is there an association between microbial exposure and food allergy? A systematic review. Pediatr Allergy Immunol. 2013;24(4):311-20 e8.
25. Sicherer SH, Sampson HA. Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. J Allergy Clin Immunol. 2014;133(2):291-307.
26. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol. 2018;141(1):41-58.
27. Gupta RS, Springston EE, Smith B, Warrier MR, Pongracic J, Holl JL. Geographic variability of childhood food allergy in the United States. Clin Pediatr (Phila). 2012;51(9):856-61.
28. Botha M, Basera W, Facey-Thomas HE, Gaunt B, Gray CL, Ramjith J, et al. Rural and urban food allergy prevalence from the South African Food Allergy (SAFFA) study. J Allergy Clin Immunol. 2019;143(2):662-8 e2.
29. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet. 2001;358(9288):1129-33.
30. Campbell B, Raherison C, Lodge CJ, Lowe AJ, Gislason T, Heinrich J, et al. The effects of growing up on a farm on adult lung function and allergic phenotypes: an international population-based study. Thorax. 2017;72(3):236-44.
31. Hong X, Wang G, Liu X, Kumar R, Tsai HJ, Arguelles L, et al. Gene polymorphisms, breastfeeding, and development of food sensitisation in early childhood. J Allergy Clin Immunol. 2011;128(2):374-81 e2.
32. Lack G. Update on risk factors for food allergy. J Allergy Clin Immunol. 2012;129(5):1187-97.
33. Perez-Escamilla R, Buccini GS, Segura-Perez S, Piwoz E. Perspective: Should Exclusive Breastfeeding Still Be Recommended for 6 Months? Adv Nutr. 2019; 1;10(6):931-943.
34. Burgess JA, Dharmage SC, Allen K, Koplin J, Garcia-Larsen V, Boyle R, et al. Age at introduction to complementary solid food and food allergy and sensitisation: A systematic review and meta-analysis. Clin Exp Allergy. 2019;49(6):754-69.
35. McGowan EC, Bloomberg GR, Gergen PJ, Visness CM, Jaffee KF, Sandel M, et al. Influence of early-life exposures on food sensitisation and food allergy in an inner-city birth cohort. J Allergy Clin Immunol. 2015;135(1):171-8.
36. Milner JD, Stein DM, McCarter R, Moon RY. Early infant multivitamin supplementation is associated with increased risk for food allergy and asthma. Pediatrics. 2004;114(1):27-32.
37. Liem JJ, Kozyrskyj AL, Huq SI, Becker AB. The risk of developing food allergy in premature or low-birth-weight children. J Allergy Clin Immunol. 2007;119(5):1203-9.
38. Mitselou N, Hallberg J, Stephansson O, Almqvist C, Melen E, Ludvigsson JF. Cesarean delivery, preterm birth, and risk of food allergy: Nationwide Swedish cohort study of more than 1 million children. J Allergy Clin Immunol. 2018;142(5):1510-4 e2.
39. Bager P, Wohlfahrt J, Westergaard T. Caesarean delivery and risk of atopy and allergic disease: meta-analyses. Clin Exp Allergy. 2008;38(4):634-42.

40. Shroba J, Rath N, Barnes C. Possible Role of Environmental Factors in the Development of Food Allergies. Clin Rev Allergy Immunol. 2018; 57(3):303-311.
41. Koplin J, Allen K, Gurrin L, Osborne N, Tang ML, Dharmage S. Is caesarean delivery associated with sensitisation to food allergens and IgE-mediated food allergy: a systematic review. Pediatr Allergy Immunol. 2008;19(8):682-7.
42. Liu AH, Jaramillo R, Sicherer SH, Wood RA, Bock SA, Burks AW, et al. National prevalence and risk factors for food allergy and relationship to asthma: results from the National Health and Nutrition Examination Survey 2005-2006. J Allergy Clin Immunol. 2010;126(4):798-806 e13.
43. Melioli G, Marcomini L, Agazzi A, Bazurro G, Tosca M, Rossi GA, et al. The IgE repertoire in children and adolescents resolved at component level: a cross-sectional study. Pediatr Allergy Immunol. 2012;23(5):433-40.
44. Soost S, Leynaert B, Almqvist C, Edenharter G, Zuberbier T, Worm M. Risk factors of adverse reactions to food in German adults. Clin Exp Allergy. 2009;39(7):1036-44.
45. Nwaru BI, Hickstein L, Panesar SS, Muraro A, Werfel T, Cardona V, et al. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. Allergy. 2014;69(1): 62-75.
45. Nwaru BI, Hickstein L, Panesar SS, Muraro A, Werfel T, Cardona V, et al. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. Allergy. 2014;69(1):62-75.
46. Woods RK, Abramson M, Bailey M, Walters EH. International prevalences of reported food allergies and intolerances. Comparisons arising from the European Community Respiratory Health Survey (ECRHS) 1991-1994. Eur J Clin Nutr. 2001;55(4):298-304.
47. Kanny G, Moneret-Vautrin DA, Flabbee J, Beaudouin E, Morisset M, Thevenin F. Population study of food allergy in France. J Allergy Clin Immunol. 2001;108(1):133-40.

Supplemental files

Supplemental methods on data collection

Questionnaires

The EuroPrevall phase I screening questionnaire was a self-administered one-page document, designed to detect subjects with and without food-related reactions. The phase II questionnaire was an extensive interviewer-administered questionnaire on symptoms and potential risk factors for FA.

Determinants

Data on evaluated childhood environmental determinants were obtained from the phase II questionnaire, and consisted of maternal age at the birth of subject, number of siblings, number of older siblings, bedroom sharing with any sibling and with older siblings, day care attendance, dog or cat ownership, growing up on a farm, growing up in the inner city, and having serious respiratory infections. In children, antibiotic use before the age of 2 years, use of reflux medication in the previous 6 months, maternal smoking during pregnancy, and maternal and paternal smoking since the birth of the subject (at least one cigar(ette) per day), were also investigated. Regarding infant diet, duration of breastfeeding, age of start of infant formula, type of infant formula (cow's milk, soy milk, hypoallergenic), age of introduction of solid foods, and vitamin D supplementation before the age of 2 years, were assessed. For adults, environmental determinants later in life were food-related occupation (ever worked in the growing, production, processing or distribution of food), smoking (ever for longer than one year), and frequency of use of indigestion medication (antacids, H2-antagonists or proton pump inhibitors). Demographic factors, consisting of age, sex, and (parental) level of education for all subjects, and gestational age at birth, birth length and weight for children, were also evaluated.

Food and inhalant allergens

Specific IgE testing was performed in phase II for 24 foods, which are often implicated in FA or frequently consumed across Europe. These foods were hen's egg, cow's milk, fish, shrimp, peanut, hazelnut, walnut, peach, apple, kiwi, melon, banana, tomato, celery, carrot, corn, lentils, soy, wheat, buckwheat, sesame seed, mustard seed, sunflower seed, and poppy seed. They were termed *priority* foods. IgE testing was also performed for 6 common inhalant allergens: birch, mugwort, grass, and *parietaria* pollen, house dust mite, and cat.

IgE testing

All serum samples were analysed in a single laboratory of the Amsterdam University Medical Centres, location AMC, Amsterdam, The Netherlands, using commercially available ImmunoCAP tests (Phadia, *currently Thermo Fisher Scientific*, Uppsala, Sweden). All sera that tested positive for at least one of the priority foods in phase II, and a random sample of non-sensitised controls, were further tested for specific food allergens using an allergen microarray assay[E1-3].

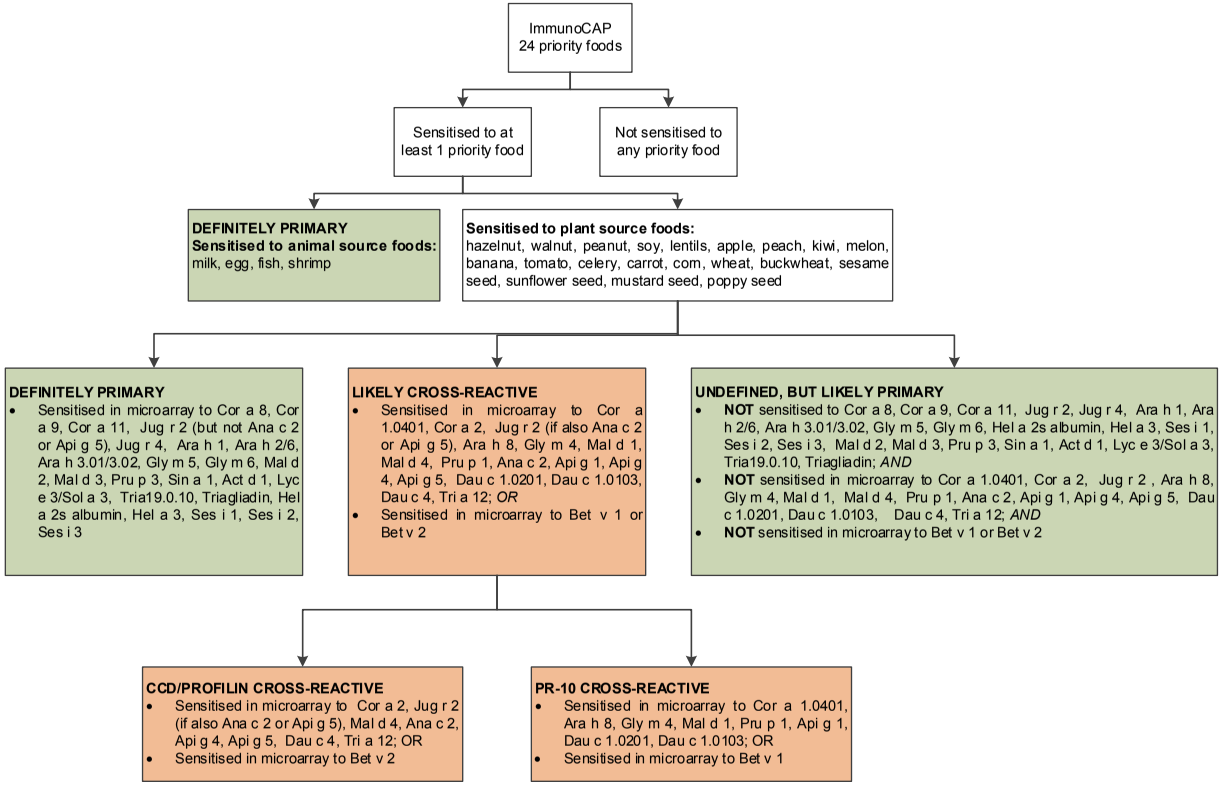

Figure S1. Classification of FS into primary and cross-reactive sensitisation

Subjects were classified into 1 *or more* of the boxes depending on their sensitisation patterns (i.e., subjects could have both primary and cross-reactive sensitisation). This is a simplified classification, designed for exploratory purposes, and subjects with cross-reactive sensitisation through food rather than pollen, or with cross-reactive sensitisation to tropomyosins (e.g., shrimp through house-dust mite), have been classified as primary sensitisation. However, aforementioned cross-reactivity patterns are much less common than pollen-related cross-reactivity, and are expected to have only limited influence on the prevalence estimates of primary FS. Green: Primary sensitisation (= definitely, or undefined but likely, primary sensitisation). Orange: cross-reactive sensitisation.

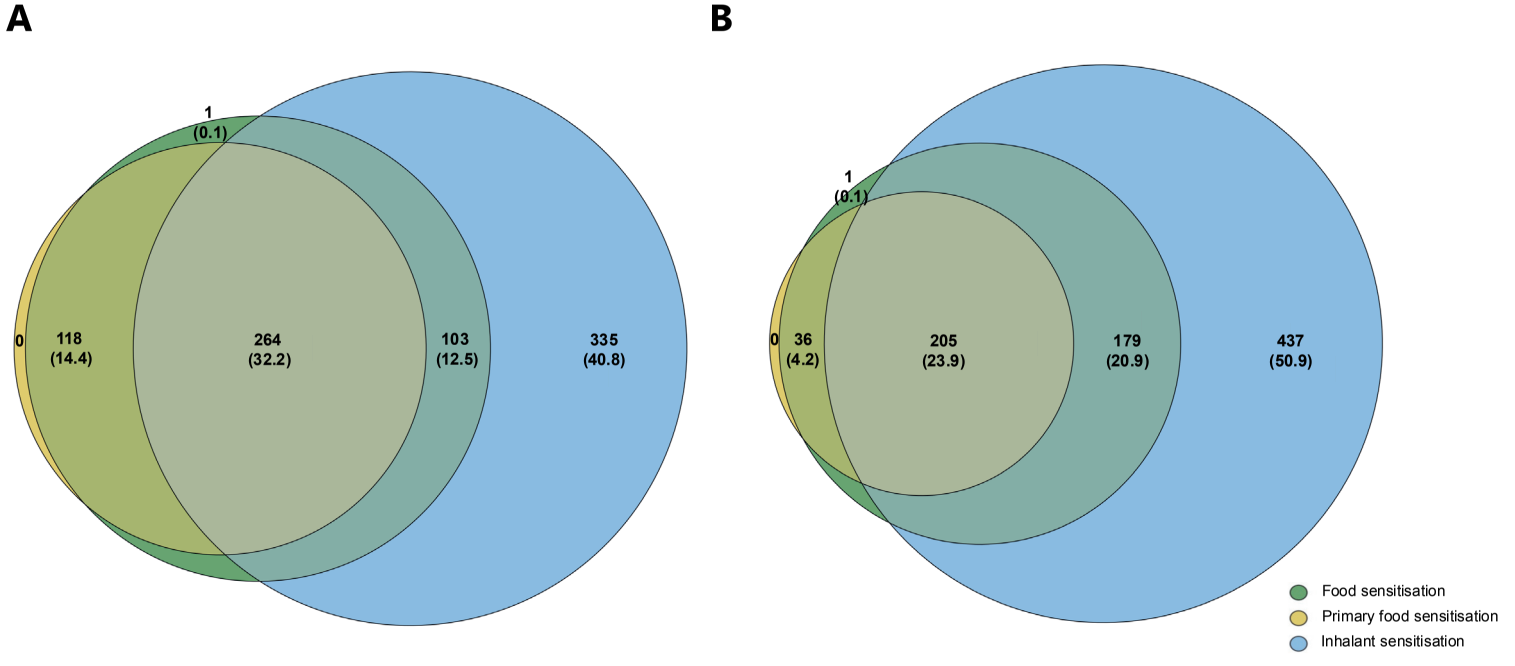

Figure S2. Relationship between FS, primary FS, and IS in (A) children and (B) adults
The numbers (%) represent individual subjects included in the study with the particular (combination of) outcome(s). Only subjects with complete data on FS, primary FS, and IS were included in these diagrams.

Table S1. Number of missing values in predictor variables

	Number of missings Children (N=2196)	Number of missings Adults (N=2185)
Demographics		
Age	0	0
Sex	0	0
Level of education	0	0
Gestational age	26	NA
Birth weight	31	NA
Birth length	76	NA
Childhood environment		
Maternal age	7	5
Number of siblings	6	9
Number of older siblings	6	9
Bedroom sharing other children	6	2
Bedroom sharing older children	2	11
Day care attendance	2	58
Farm environment	0	0
Inner city environment	0	0
Pet dog	1	0
Pet cat	1	0
Serious respiratory infection	1	186
Use of antibiotics	2	NA
Maternal smoking pregnancy	3	NA
Maternal smoking since birth	2	NA
Paternal smoking since birth	2	NA
Reflux medication last 6 months	0	NA
Infant diet		
Vitamin D supplementation	123	NA
Breastfeeding duration	30	NA
Cow's milk infant formula	22	NA
Soy milk infant formula	26	NA
Hypoallergenic infant formula	25	NA
Age start infant formula	31	NA
Age introduction solid foods	50	NA
Adult environment		
Smoking	NA	0
Food related work	NA	0
Indigestion medication currently	NA	0
Variables for model adjustment		
Comorbid atopy	50	103
Parental food allergy	233	NA
Sensitisation to pollen	32	44
Sensitisation to HDM or cat	32	44
Primary food sensitisation	1	0
Centre	0	0

Values for these missing data were estimated using multiple imputation procedures, for which all of the above determinants were included as covariates, along with the outcome food sensitisation. *NA, not available.*

Table S2. Comparison of subjects with and without available food serology

	Children			Adults		
	Food serology available (N=2196)	**Food serology missing (N=130)**	**p**	**Food serology available (N=2185)**	**Food serology missing (N=71)**	**p**
Age in years; *mean (±SD)*	8.9 (±1.0)	8.9 (±0.9)	0.937	38.3 (±9.6)	38.1 (±9.0)	0.842
Sex						
Male	1096 (49.9)	61 (46.9)	0.508	932 (42.7)	20 (28.2)	**0.015**
Female	1100 (50.1)	69 (53.1)		1253 (57.3)	51 (71.8)	
Level of education*						
High	938 (42.7)	61 (46.9)	0.348	1436 (65.7)	44 (62.0)	0.513
Low	1257 (57.3)	61 (46.9)		749 (34.3)	27 (38.0)	
Allergic asthma	416 (18.9)	22 (16.9)	0.567	323 (14.8)	6 (8.5)	0.137
Allergic rhinitis	710 (32.3)	43 (33.1)	0.860	1093 (50.0)	34 (47.9)	0.810
Atopic dermatitis	833 (37.9)	56 (43.1)	0.241	393 (18.0)	10 (14.1)	0.398
Family atopy	1732 (81.9)	102 (81.6)	0.935	1411 (73.7)	36 (63.2)	0.075
Parental food allergy	505 (25.7)	31 (27.0)	0.769	NA	NA	NA
Symptoms to any foods	917 (41.8)	53 (40.8)	0.824	1121 (51.3)	31 (43.7)	0.205
Symptoms to priority foods	661 (30.1)	30 (23.1)	0.089	744 (34.1)	18 (25.4)	0.127
Centre						
Zurich	304 (13.8)	26 (20.0)	**<0.001**	482 (22.1)	22 (31.0)	**<0.001**
Madrid	278 (12.7)	22 (16.9)		309 (14.1)	5 (7.0)	
Athens	155 (7.1)	27 (20.8)		67 (3.1)	35 (49.3)	
Utrecht	398 (18.1)	23 (18.5)		476 (21.8)	4 (5.6)	
Lodz	453 (20.6)	6 (4.6)		378 (17.3)	5 (7.0)	
Reykjavik	457 (20.8)	12 (9.2)		473 (21.6)	0 (0.0)	
Vilnius	151 (6.9)	13 (10.0)		NA	NA	

Results presented in N (%) unless otherwise specified. **Bold** indicates p<0.05. *For children: high level of education parents. *NA, not available.*

Table S3. Comparison model Ⅲ to model Ⅲ adjusted for comorbid atopy and inhalant sensitisation

	Model Ⅲ				**Model Ⅲ +comorbid atopy[a]**				**Model Ⅲ+comorbid atopy+inhalant sensitisation[b]**			
	Children		**Adults**		**Children**		**Adults**		**Children**		**Adults**	
	OR [95%-CI]	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**
Age per year	1.01 [0.91-1.13]	0.85	**0.97 [0.96-0.98]**	**<0.001**	1.02 [0.90-1.16]	0.85	**0.97 [0.96-0.99]**	**<0.001**	0.96 [0.84-1.10]	0.55	0.99 [0.97-1.01]	0.21
Male sex	1.01 [0.82-1.25]	0.92	**1.39 [1.12-1.73]**	**<0.001**	1.01 [0.79-1.28]	0.81	**1.45 [1.16-1.81]**	**<0.001**	0.64 [0.49-0.84]	**<0.001**	**1.34 [1.01-1.77]**	**0.04**
High level of education*	1.02 [0.80-1.29]	0.88	0.86 [0.67-1.09]	0.21	1.02 [0.79-1.36]	1.00	0.84 [0.65-1.07]	0.16	0.96 [0.72-1.29]	0.80	0.76 [0.56-1.04]	0.09
Gestational age per week	**0.93 [0.87-0.99]**	**0.01**	NA	NA	**0.93 [0.88-0.99]**	**0.02**	NA	NA	**0.92 [0.86-0.99]**	**0.03**	NA	NA
Birth length per centimetre	1.00 [1.00-1.00]	0.97	NA	NA	1.01 [0.96-1.05]	0.71	NA	NA	1.01 [0.96-1.06]	0.75	NA	NA
Birth weight per 100 grams	1.01 [0.97-1.06]	0.65	NA	NA	1.00 [1.00-1.00]	0.93	NA	NA	1.00 [1.00-1.00]	0.85	NA	NA
Maternal age per year	0.99 [0.97-1.01]	0.33	1.01 [0.99-1.03]	0.45	0.99 [0.96-1.01]	0.32	1.01 [0.98-1.03]	0.50	0.99 [0.96-1.02]	0.46	1.00 [0.97-1.03]	0.98
Number of siblings		0.28		0.74		0.36		0.50		0.89		0.51
1	0.86 [0.63-1.18]		1.22 [0.75-1.97]		0.85 [0.62-1.17]		1.38 [0.85-2.23]		1.03 [0.70-1.51]		0.92 [0.50-1.69]	
2	1.09 [0.73-1.64]		1.34 [0.78-2.29]		1.06 [0.71-1.59]		1.52 [0.89-2.61]		1.04 [0.63-1.73]		1.25 [0.63-2.47]	
3 or more	0.76 [0.40-1.44]		1.35 [0.75-2.42]		0.77 [0.40-1.47]		1.49 [0.83-2.68]		0.80 [0.36-1.76]		1.15 [0.55-2.41]	
Number of older siblings		0.62		0.75		0.65		0.77		0.70		0.18
1	0.85 [0.63-1.15]		1.17 [0.85-1.62]		0.86 [0.64-1.16]		1.17 [0.84-1.61]		0.89 [0.62-1.28]		1.56 [1.04-2.35]	
2	1.02 [0.63-1.18]		1.21 [0.78-1.88]		1.02 [0.63-1.65]		1.21 [0.78-1.88]		1.21 [0.67-2.18]		1.34 [0.76-2.35]	
3 or more	0.77 [0.31-1.95]		1.22 [0.69-2.14]		0.75 [0.29-1.90]		1.20 [0.68-2.11]		1.06 [0.35-3.17]		1.23 [0.60-2.54]	
Bedroom sharing with other children	0.77 [0.53-1.13]†	0.18	0.84 [0.62-1.14]‡	0.26	0.76 [0.51-1.11]†	0.15	0.84 [0.62-1.13]‡	0.25	1.02 [0.63-1.63]†	0.95	0.95 [0.65-1.40]‡	0.79
Bedroom sharing with older children	1.19 [0.79-1.80]†	0.40	0.75 [0.52-1.06]‡	0.11	1.21 [0.80-1.83]†	0.37	0.77 [0.54-1.10]‡	0.14	1.08 [0.65-1.79]†	0.78	0.76 [0.48-1.19]‡	0.23
Day care attendance	0.82 [0.64-1.05]†	0.12	1.02 [0.80-1.30]‡	0.89	0.82 [0.64-1.06]†	0.13	1.01 [0.54-1.10]‡	0.97	0.84 [0.62-1.13]†	0.24	1.10 [0.80-1.50]‡	0.56
Farm environment	1.06 [0.21-5.36]†	0.95	0.73 [0.40-1.35]‡	0.32	1.09 [0.21-5.56]†	0.92	0.75 [0.40-1.39]‡	0.35	1.76 [0.27-11.39]†	0.55	1.15 [0.53-2.51]‡	0.72
Inner city environment	0.94 [0.76-1.17]†	0.60	1.01 [0.79-1.29]‡	0.93	0.96 [0.77-1.19]†	0.69	0.97 [0.76-1.25]‡	0.84	1.02 [0.79-1.34]†	0.86	1.09 [0.79-1.50]‡	0.60

Table S3. Comparison model Ⅲ to model Ⅲ adjusted for comorbid atopy and inhalant sensitisation (continued)

	Model Ⅲ				**Model Ⅲ +comorbid atopy[a]**				**Model Ⅲ+comorbid atopy+inhalant sensitisation[b]**			
	Children		**Adults**		**Children**		**Adults**		**Children**		**Adults**	
	OR [95%-CI]	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**
Pet dog	**0.65 [0.48-0.90]†**	**0.01**	0.94 [0.70-1.27]‡	0.61	**0.65 [0.47-0.89]†**	**0.01**	0.94 [0.74-1.21]‡	0.65	0.81 [0.55-1.18]†	0.27	1.12 [0.82-1.52]‡	0.48
Pet cat	1.04 [0.77-1.40]†	0.79	0.87 [0.68-1.10]‡	0.24	1.02 [0.76-1.38]†	0.89	0.89 [0.70-1.13]‡	0.35	1.26 [0.88-1.80]†	0.21	1.06 [0.78-1.43]‡	0.71
Serious respiratory infection	1.11 [0.86-1.45]†	0.42	0.88 [0.62-1.25]‡	0.47	1.10 [0.84-1.43]†	0.50	0.86 [0.60-1.23]‡	0.40	1.33 [0.96-1.83]†	0.09	0.90 [0.58-1.41]‡	0.66
Use of antibiotics	1.14 [0.89-1.45]†	0.29	NA	NA	1.10 [0.86-1.40]†	0.46	NA	NA	1.07 [0.79-1.43]†	0.67	NA	NA
Maternal smoking during pregnancy	0.74 [0.51-1.08]	0.12	NA	NA	0.74 [0.51-1.07]	0.11	NA	NA	0.71 [0.45-1.13]	0.15	NA	NA
Maternal smoking since birth	1.12 [0.84-1.48]	0.45	NA	NA	1.13 [0.85-1.50]	0.41	NA	NA	1.35 [0.95-1.91]	0.09	NA	NA
Paternal smoking since birth	0.97 [0.77-1.22]	0.78	NA	NA	0.97 [0.77-1.23]	0.82	NA	NA	0.86 [0.65-1.13]	0.27	NA	NA
Reflux medication in last 6 months	0.61 [0.17-2.19]	0.45	NA	NA	0.56 [0.16-2.02]	0.38	NA	NA	0.70 [0.15-3.30]	0.65	NA	NA
Vitamin D supplementation	1.05 [0.75-1.47]†	0.77	NA	NA	1.03 [0.74-1.45]†	0.85	NA	NA	1.01 [0.67-1.53]†	0.96	NA	NA
Breastfeeding duration		0.29				0.40				0.27		
≤4 months	1.20 [0.82-1.75]		NA	NA	1.17 [0.80-1.72]		NA	NA	1.30 [0.81-2.08]		NA	NA
4-6 months	1.20 [0.75-1.94]		NA	NA	1.19 [0.74-1.93]	0.28	NA	NA	1.28 [0.71-2.31]	0.61	NA	NA
>6 months	0.91 [0.57-1.46]		NA	NA	0.89 [0.55-1.42]		NA	NA	1.11 [0.62-1.97]		NA	NA
Cow's milk infant formula	0.85 [0.58-1.24]	0.39	NA	NA	0.83 [0.57-1.21]		NA	NA	0.77 [0.48-1.23]		NA	NA
Soy milk infant formula	1.35 [0.89-2.05]	0.16	NA	NA	1.29 [0.84-1.96]	0.23	NA	NA	1.17 [0.69-1.98]	0.56	NA	NA
Hypoallergenic infant formula	**1.51 [1.06-2.15]**	**0.02**	NA	NA	**1.44 [1.01-2.05]**	**0.04**	NA	NA	1.27 [0.82-1.96]	0.29	NA	NA
Age start infant formula		0.33				0.31				0.59		
0-4 months	1.02 [0.60-1.72]		NA	NA	1.05 [0.62-1.77]		NA	NA	1.31 [0.70-2.48]		NA	NA
4-6 months	1.04 [0.60-1.79]		NA	NA	1.05 [0.61-1.82]		NA	NA	1.25 [0.64-2.45]		NA	NA
6-11 months	1.10 [0.66-1.82]		NA	NA	1.12 [0.67-1.86]		NA	NA	1.55 [0.82-2.91]		NA	NA
≥11 months	1.79 [0.94-3.40]		NA	NA	1.84 [0.97-3.51]		NA	NA	1.73 [0.78-3.84]		NA	NA

Table S3. Comparison model III to model III adjusted for comorbid atopy and inhalant sensitisation (continued)

	Model III				**Model III +comorbid atopy[a]**				**Model III+comorbid atopy+inhalant sensitisation[b]**			
	Children		**Adults**		**Children**		**Adults**		**Children**		**Adults**	
	OR [95%-CI]	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**
Age introduction solid foods		0.19				0.17				0.82		
4-6 months	1.37 [1.00-1.86]		NA	NA	1.39 [1.02-1.89]		NA	NA	1.18 [0.81-1.71]		NA	NA
6-11 months	1.22 [0.86-1.71]		NA	NA	1.22 [0.87-1.72]		NA	NA	1.12 [0.75-1.69]		NA	NA
≥11 months	1.45 [0.93-2.27]		NA	NA	1.43 [0.92-2.24]		NA	NA	1.07 [0.61-1.86]		NA	NA
Smoking	NA	NA	0.99 [0.79-1.24]	0.94	NA	NA	1.03 [0.82-1.29]	0.81	NA	NA	1.20 [0.90-1.61]	0.22
Food-related occupation	NA	NA	0.83 [0.64-1.07]	0.15	NA	NA	0.80 [0.62-1.05]	0.11	NA	NA	0.80 [0.57-1.11]	0.18
Indigestion medication currently				0.59				0.63				0.16
Yes, <1x/month	NA	NA	1.30 [0.85-1.97]		NA	NA	1.21 [0.79-1.85]		NA	NA	1.43 [0.84-2.44]	
Yes, <1x/week	NA	NA	1.10 [0.46-2.60]		NA	NA	1.01 [0.42-2.42]		NA	NA	1.06 [0.35-3.20]	
Yes, ≥ 1x/week	NA	NA	0.84 [0.46-1.54]		NA	NA	0.76 [0.41-1.41]		NA	NA	1.17 [0.56-2.42]	
Comorbid atopy	NA	NA	NA	NA	**2.02 [1.30-3.15]**	**<0.001**	**3.55 [2.22-5.66]**	**<0.001**	1.40 [0.85-2.30]	0.18	1.60 [0.92-2.79]	0.10
Parental food allergy	NA	NA	NA	NA	1.12 [0.87-1.44]	0.37	NA	NA	0.89 [0.65-1.21]	0.46	NA	NA
Positive serology to pollen	NA	NA	NA	NA	NA	NA	NA	NA	**13.15 [9.92-17.44]**	**<0.001**	**13.10 [9.67-17.74]**	**<0.001**
Positive serology to HDM or cat	NA	NA	NA	NA	NA	NA	NA	NA	**2.79 [2.08-3.74]**	**<0.001**	**5.35 [3.96-7.22]**	**<0.001**

Analyses were adjusted for centre. [a]Adjusted for comorbid atopy (asthma, allergic rhinitis or atopic dermatitis in subject or first degree family member). [b]Adjusted for comorbid atopy and inhalant sensitisation (sIgE to birch, grass, mugwort, parietaria, HDM or cat). All p-values for continuous variables were based on the two-sample t-test, and those for categorical variables on the chi-square test. **Bold** indicates p<0.05 *For children: high level of education parents. [†]Before the age of 2 years. [‡]Before the age of 5 years. Reference: "number of (older) siblings" = 0; "indigestion medication" = no or <1x/year; "breastfeeding duration" = never; "age start infant formula" = never; "age introduction solid food" = 0-4 months. *HDM, house dust mite; OR, odds ratio; CI, confidence interval; NA, not available.*

Table S4. Predictors for food sensitisation compared to predictors for inhalant sensitisation and primary food sensitisation

	Food sensitisation				**Inhalant sensitisation**				**Primary food sensitisation**			
	Children		**Adults**		**Children**		**Adults**		**Children**		**Adults**	
	OR [95%-CI]	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**
Age per year	1.01 [0.91-1.13]	0.85	**0.97 [0.96-0.98]**	**<0.001**	**1.11 [1.00-1.22]**	**0.05**	**0.96 [0.95-0.97]**	**<0.001**	1.00 [0.88-1.12]	0.93	**0.96 [0.95-0.98]**	**<0.001**
Male sex	1.01 [0.82-1.25]	0.92	**1.39 [1.12-1.73]**	**<0.001**	**1.62 [1.34-1.96]**	**<0.001**	**1.39 [1.16-1.68]**	**<0.001**	0.99 [0.79-1.25]	0.93	**1.46 [1.10-1.92]**	**0.01**
High level of education*	1.02 [0.80-1.29]	0.88	0.86 [0.67-1.09]	0.21	1.10 [0.89-1.36]	0.40	0.99 [0.80-1.22]	0.93	0.93 [0.72-1.20]	0.59	0.89 [0.66-1.21]	0.47
Gestational age per week	**0.93 [0.87-0.99]**	**0.01**	NA	NA	0.96 [0.91-1.02]	0.15	NA	NA	0.96 [0.90-1.02]	0.16	NA	NA
Birth length per centimetre	1.00 [1.00-1.00]	0.97	NA	NA	1.00 [1.00-1.00]	0.15	NA	NA	1.00 [1.00-1.00]	0.57	NA	NA
Birth weight per 100 grams	1.01 [0.97-1.06]	0.65	NA	NA	0.99 [0.97-1.02]	0.52	NA	NA	1.02 [0.97-1.07]	0.43	NA	NA
Maternal age per year	0.99 [0.97-1.01]	0.33	1.01 [0.99-1.03]	0.45	0.99 [0.97-1.02]	0.61	**1.02 [1.00-1.04]**	**0.03**	0.99 [0.97-1.02]	0.46	1.01 [0.98-1.04]	0.66
Number of siblings		0.28		0.74		0.16		0.22		0.29		0.78
1	0.86 [0.63-1.18]		1.22 [0.75-1.97]		0.87 [0.66-1.16]		1.50 [1.01-2.25]		0.91 [0.65-1.28]		1.20 [0.65-2.23]	
2	1.09 [0.73-1.64]		1.34 [0.78-2.29]		1.18 [0.82-1.70]		1.37 [0.87-2.16]		1.22 [0.79-1.89]		1.40 [0.70-2.78]	
3 or more	0.76 [0.40-1.44]		1.35 [0.75-2.42]		0.93 [0.53-1.63]		1.51 [0.93-2.47]		0.87 [0.43-1.73]		1.30 [0.61-2.79]	
Number of older siblings		0.62		0.75		0.76		0.63		0.79		0.19
1	0.85 [0.63-1.15]		1.17 [0.85-1.62]		0.92 [0.71-1.20]		0.85 [0.65-1.12]		0.85 [0.61-1.17]		1.47 [0.99-2.18]	
2	1.02 [0.63-1.18]		1.21 [0.78-1.88]		0.81 [0.53-1.26]		0.95 [0.66-1.38]		0.91 [0.54-1.52]		1.22 [0.70-2.13]	
3 or more	0.77 [0.31-1.95]		1.22 [0.69-2.14]		0.99 [0.47-2.10]		0.84 [0.53-1.34]		0.90 [0.34-2.35]		1.73 [0.86-3.46]	
Bedroom sharing with other children	0.77 [0.53-1.13]†	0.18	0.84 [0.62-1.14]‡	0.26	**0.66 [0.46-0.95]**†	**0.02**	0.81 [0.62-1.04]‡	0.10	0.88 [0.59-1.33]†	0.54	1.03 [0.70-1.50]‡	0.89
Bedroom sharing with older children	1.19 [0.79-1.80]†	0.40	0.75 [0.52-1.06]‡	0.11	1.19 [0.81-1.74]†	0.37	0.86 [0.64-1.16]‡	0.32	1.14 [0.73-1.77]†	0.56	**0.62 [0.41-0.96]**‡	**0.03**
Day care attendance	0.82 [0.64-1.05]†	0.12	1.02 [0.80-1.30]‡	0.89	0.95 [0.76-1.18]†	0.63	0.90 [0.73-1.11]‡	0.35	0.88 [0.68-1.15]†	0.36	0.91 [0.67-1.24]‡	0.57
Farm environment	1.06 [0.21-5.36]†	0.95	0.73 [0.40-1.35]‡	0.32	0.22 [0.03-1.88]†	0.17	**0.43 [0.25-0.73]**‡	**<0.001**	1.48 [0.30-7.47]†	0.63	0.90 [0.44-1.84]‡	0.77
Inner city environment	0.94 [0.76-1.17]†	0.60	1.01 [0.79-1.29]‡	0.93	0.88 [0.72-1.07]†	0.20	0.84 [0.68-1.04]‡	0.11	0.96 [0.76-1.22]†	0.76	1.17 [0.86-1.59]‡	0.32

Table S4. Predictors for food sensitisation compared to predictors for inhalant sensitisation and primary food sensitisation (continued)

	Food sensitisation				**Inhalant sensitisation**				**Primary food sensitisation**			
	Children		**Adults**		**Children**		**Adults**		**Children**		**Adults**	
	OR [95%-CI]	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**
Pet dog	**0.65 [0.48-0.90]†**	**0.01**	0.94 [0.70-1.27]‡	0.61	**0.73 [0.56-0.96]†**	**0.02**	0.95 [0.77-1.18]‡	0.66	**0.68 [0.48-0.96]†**	**0.03**	1.02 [0.75-1.40]‡	0.88
Pet cat	1.04 [0.77-1.40]†	0.79	0.87 [0.68-1.10]‡	0.24	0.91 [0.70-1.19]†	0.50	**0.80 [0.65-0.98]‡**	**0.03**	0.98 [0.71-1.36]†	0.92	1.02 [0.76-1.38]‡	0.88
Serious respiratory infection	1.11 [0.86-1.45]†	0.42	0.88 [0.62-1.25]‡	0.47	0.81 [0.63-1.03]†	0.08	0.94 [0.70-1.27]‡	0.71	1.16 [0.87-1.54]†	0.30	1.37 [0.91-2.05]‡	0.13
Use of antibiotics	1.14 [0.89-1.45]†	0.29	NA	NA	1.08 [0.87-1.35]†	0.46	NA	NA	1.12 [0.86-1.45]†	0.40	NA	NA
Maternal smoking during pregnancy	0.74 [0.51-1.08]	0.12	NA	NA	0.87 [0.62-1.22]	0.42	NA	NA	0.85 [0.57-1.27]	0.43	NA	NA
Maternal smoking since birth	1.12 [0.84-1.48]	0.45	NA	NA	0.86 [0.66-1.11]	0.24	NA	NA	1.06 [0.78-1.44]	0.73	NA	NA
Paternal smoking since birth	0.97 [0.77-1.22]	0.78	NA	NA	1.11 [0.90-1.36]	0.33	NA	NA	0.95 [0.74-1.22]	0.69	NA	NA
Reflux medication in last 6 months	0.61 [0.17-2.19]	0.45	NA	NA	0.63 [0.20-2.00]	0.43	NA	NA	0.85 [0.24-3.05]	0.81	NA	NA
Vitamin D supplementation	1.05 [0.75-1.47]†	0.77	NA	NA	0.95 [0.70-1.29]†	0.74	NA	NA	0.99 [0.69-1.42]†	0.96	NA	NA
Breastfeeding duration		0.29				0.23				0.54		
≤4 months	1.20 [0.82-1.75]		NA	NA	1.24 [0.88-1.75]		NA	NA	1.14 [0.75-1.72]		NA	NA
4-6 months	1.20 [0.75-1.94]		NA	NA	1.43 [0.93-2.21]		NA	NA	1.34 [0.81-2.23]		NA	NA
>6 months	0.91 [0.57-1.46]		NA	NA	1.10 [0.72-1.68]		NA	NA	1.06 [0.64-1.75]		NA	NA
Cow's milk infant formula	0.85 [0.58-1.24]	0.39	NA	NA	1.08 [0.76-1.53]	0.68	NA	NA	0.94 [0.63-1.42]	0.78	NA	NA
Soy milk infant formula	1.35 [0.89-2.05]	0.16	NA	NA	1.27 [0.86-1.87]	0.23	NA	NA	1.35 [0.86-2.13]	0.19	NA	NA
Hypoallergenic infant formula	**1.51 [1.06-2.15]**	**0.02**	NA	NA	**1.62 [1.17-2.25]**	**<0.001**	NA	NA	1.44 [0.98-2.11]	0.06	NA	NA
Age start infant formula		0.33				0.08				0.93		
0-4 months	1.02 [0.60-1.72]		NA	NA	0.83 [0.52-1.33]		NA	NA	1.06 [0.61-1.86]		NA	NA
4-6 months	1.04 [0.60-1.79]		NA	NA	0.69 [0.42-1.15]		NA	NA	1.04 [0.58-1.88]		NA	NA
6-11 months	1.10 [0.66-1.82]		NA	NA	0.70 [0.44-1.10]		NA	NA	1.10 [0.63-1.90]		NA	NA
≥11 months	1.79 [0.94-3.40]		NA	NA	1.31 [0.74-2.33]		NA	NA	1.32 [0.64-2.71]		NA	NA

Table S4. Predictors for food sensitisation compared to predictors for inhalant sensitisation and primary food sensitisation (continued)

	Food sensitisation				**Inhalant sensitisation**				**Primary food sensitisation**			
	Children		**Adults**		**Children**		**Adults**		**Children**		**Adults**	
	OR [95%-CI]	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**	**OR [95%-CI]**	**p**
Age introduction solid foods		0.19				0.17				0.82		
4-6 months	1.37 [1.00-1.86]		NA	NA	1.39 [1.02-1.89]		NA	NA	1.18 [0.81-1.71]		NA	NA
6-11 months	1.22 [0.86-1.71]		NA	NA	1.22 [0.87-1.72]		NA	NA	1.12 [0.75-1.69]		NA	NA
≥11 months	1.45 [0.93-2.27]		NA	NA	1.43 [0.92-2.24]		NA	NA	1.07 [0.61-1.86]		NA	NA
Smoking	NA	NA	0.99 [0.79-1.24]	0.94	NA	NA	0.89 [0.74-1.08]	0.24	NA	NA	**1.35 [1.01-1.80]**	**0.04**
Food-related occupation	NA	NA	0.83 [0.64-1.07]	0.15	NA	NA	0.97 [0.78-1.21]	0.80	NA	NA	0.77 [0.55-1.08]	0.13
Indigestion medication currently				0.59				0.91				0.09
Yes, <1x/month	NA	NA	1.30 [0.85-1.97]		NA	NA	1.05 [0.72-1.52]		NA	NA	1.75 [1.09-2.82]	
Yes, <1x/week	NA	NA	1.10 [0.46-2.60]		NA	NA	1.10 [0.53-2.28]		NA	NA	1.77 [0.71-4.47]	
Yes, ≥ 1x/week	NA	NA	0.84 [0.46-1.54]		NA	NA	0.87 [0.54-1.40]		NA	NA	1.21 [0.62-2.35]	

Analyses were adjusted for centre. All p-values for continuous variables were based on the two-sample t-test, and those for categorical variables on the chi-square test. **Bold** indicates p<0.05 *For children: high level of education parents. †Before the age of 2 years. ‡Before the age of 5 years. Reference: "number of (older) siblings" = 0; "indigestion medication" = no or <1x/year; "breastfeeding duration" = never; "age start infant formula" = never; "age introduction solid food" = 0-4 months. *OR, odds ratio; CI, confidence interval; NA, not available.*

References supplemental files

E1. Kummeling I, Mills EN, Clausen M, Dubakiene R, Perez CF, Fernandez-Rivas M, et al. The EuroPrevall surveys on the prevalence of food allergies in children and adults: background and study methodology. Allergy. 2009;64(10):1493-7.

E2. Burney PG, Potts J, Kummeling I, Mills EN, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitisation in European adults. Allergy. 2014;69(3):365-71.

E3. Hiller R, Laffer S, Harwanegg C, Huber M, Schmidt WM, Twardosz A, et al. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. FASEB J. 2002;16(3):414-6.

PART

Food allergy in the presenting patient

prediction, patient profiles and pollen-related food allergy

Chapter 5

Predicting food allergy: the value of patient history reinforced

Allergy. 2020. In press.

Sarah A. Lyons, André C. Knulst, Peter G.J. Burney, Montserrat Fernández-Rivas, Barbara K. Ballmer-Weber, Laura Barreales, Christian Bieli, Michael Clausen, Ruta Dubakiene, Cristina Fernández-Pérez, Monika Jedrzejczak-Czechowicz, Marek L. Kowalski, Ischa Kummeling, Tanya Kralimarkova, Tihomir B. Mustakov, Harmieke van Os-Medendorp, Nikolaos G. Papadopoulos, Todor A. Popov, James Potts, Serge A. Versteeg, Paraskevi Xepapadaki, Paco M.J. Welsing, E.N. Clare Mills, Ronald van Ree, Thuy-My Le

Abstract

Background
EAACI guidelines emphasise the importance of patient history in diagnosis of food allergy (FA), and the need for studies investigating its value, using standardised allergy-focused questionnaires.

Objective
To determine the contribution of reaction characteristics, allergic comorbidities, and demographics for prediction of FA in individuals experiencing food-related adverse reactions.

Methods
Adult and school-age participants in the standardised EuroPrevall population surveys, with self-reported FA, were included. Penalised multivariable regression was used to assess the association of patient history determinants with 'probable' FA, defined as a food-specific case history supported by relevant IgE sensitisation.

Results
In adults (N=844), reproducibility of reaction (OR 1.35 [95%-CI 1.29-1.41]), oral allergy symptoms (4.46 [4.19-4.75]), allergic rhinitis comorbidity (2.82 [2.68-2.95]), asthma comorbidity (1.38 [1.30-1.46]), and male sex (1.50 [1.41-1.59]), were positively associated with probable FA. Gastrointestinal symptoms (0.88 [0.85-0.91]) made probable FA less likely. The AUC of a model combining all selected predictors was 0.85 after cross-validation. In children (N=670), oral allergy symptoms (2.26 [2.09-2.44]) and allergic rhinitis comorbidity (1.47 [CI 1.39-1.55]) contributed most to prediction of probable FA, with a combined cross-validation-based AUC of 0.73. When focusing on plant foods, the dominant source of FA in adults, the paediatric model also included gastrointestinal symptoms (inverse association), and the AUC increased to 0.81.

Conclusions
In both adults and school-age children from the general population, reporting of oral allergy symptoms, and allergic rhinitis comorbidity, appear to be the strongest predictors of probable FA. Patient history particularly allows for good discrimination between presence and absence of probable plant FA.

Introduction

Typical food allergies (FAs) are IgE-mediated. In some parts of Europe, the prevalence of self-reported FA to commonly incriminated foods is as high as 19% in adults and 25% in school-age children from the general population.[1, 2] The majority of these self-reported adverse reactions to foods are however not attributed to IgE: the prevalence of probable FA, defined as a food-specific case history supported by relevant IgE sensitisation, is much lower than the prevalence of self-reported FA.[1-3]

A key tool available to all physicians for assessing the likelihood of FA, is patient history. FA guidelines from the European Academy of Allergy and Clinical Immunology (EAACI) acknowledge the importance of patient history in the diagnosis of FA, but also highlight that studies evaluating the accuracy of predictions using standardised allergy-focused history questionnaires are lacking, as well as studies modelling the use of history to predict FA.[4] Current evidence is based on expert opinion.[4, 5] Therefore, the EAACI guidelines have assigned high priority to clinical studies investigating this knowledge gap.[4]

The data collected using well-standardised questionnaires in the EU-funded multicentre EuroPrevall project, designed to evaluate FA across Europe, provide a unique opportunity to investigate the value of information available from patient history for predicting FA. The objective of this study was to ascertain which reaction characteristics, allergic comorbidities, and demographic factors, contribute to prediction of probable FA in adults and school-age children reporting food-related symptoms.

Methods

Study design, setting and subjects

As part of the EuroPrevall project, data were collected between 2005 and 2009 from 20- to 54-year-old and 7- to 10-year-old individuals randomly sampled from the general population of socio-economically and climatically different regions in Europe. The detailed methodology of this study is described elsewhere.[2, 3, 6] The study population for the current study consisted of subjects with self-reported FA from Athens (Greece), Lodz (Poland), Madrid (Spain), Reykjavik (Iceland), Utrecht (the Netherlands), Vilnius (Lithuania), and Zurich (Switzerland). Subjects responded to a short screening questionnaire on adverse reactions to food, symptoms, and incriminated foods, in phase I of the EuroPrevall study. Subjects were further evaluated in phase II if they indicated that they had symptoms to one of 24 foods frequently consumed or commonly implicated in food allergic reactions across Europe (so-called *priority foods:* cow's milk, hen's egg, fish, shrimp, peanut, hazelnut,

peach, apple, celery, walnut, soy, wheat, buckwheat, kiwi fruit, corn, carrot, tomato, melon, banana, lentils, sesame seed, mustard seed, sunflower seed, and poppy seed). Phase II consisted of an extensive questionnaire and blood sampling to test for presence of IgE against priority foods. All subjects with self-reported symptoms to one of the 24 priority foods, a completed phase II questionnaire, and IgE serology testing, were included in this study.[2, 3] The local ethical committees of all participating centres approved this study, and informed consent was obtained from all participants.

Data collection

The outcome, probable FA, was considered present in subjects with IgE sensitisation corresponding to a self-reported adverse reaction to at least one of the 24 priority foods. Commercially available ImmunoCAP tests (Phadia, *currently Thermo Fisher Scientific*, Uppsala, Sweden) were used to measure serum sIgE levels, and a value ≥0.35 kU_A/l was considered positive. All serology testing was performed at a single laboratory in the Amsterdam University Medical Centres (Location AMC, Amsterdam, the Netherlands).

Evaluated predictors were: reaction characteristics (time until onset, reproducibility of the reaction, oral allergy symptoms [OAS], skin symptoms, gastrointestinal symptoms, rhinoconjunctivitis, respiratory symptoms, and cardiovascular symptoms), allergic comorbidities (asthma, allergic rhinitis [AR], atopic dermatitis [AD]), demographic factors (age, sex, [parental] level of education), and (parental) smoking. The predictor information was obtained from both the phase I and phase II questionnaires, which were enriched versions of well-standardised allergy questionnaires,[7] with a specific focus on reactions to the priority foods. The phase I questionnaire was self-administered, the phase II questionnaire was conducted by trained interviewers.

Data analysis

Analyses were performed separately for adults and children. Differences between subjects with and without probable FA were described, and analysed using the chi-square test, two-sample T-test or Mann-Whitney U test, as appropriate for the variable's distribution. After obtaining crude odds ratios (ORs) for each of the evaluated predictors through univariable logistic regression, multivariable logistic regression was performed with all predictors to determine adjusted ORs. Because the probability of probable FA is known to differ per centre,[2, 3] centre was included as a covariate in the analysis. The Lithuanian site Vilnius was only included in the paediatric population, as very few (N=4) Lithuanian adults with self-reported FA participated in phase II.[3]

In order to present a parsimonious model with the most discriminative combination of the evaluated predictors for probable FA, and to avoid overfitting, Least Absolute Shrinkage and Selection Operator (Lasso) regression was applied. Lasso regression is a form of penalised regression, in which only the most contributive variables are selected, and shrinkage of regression coefficients is applied through cross-validation, to arrive at a more generalisable model.[8] The area under the curve (AUC) of the receiver-operating characteristic (ROC) was calculated to evaluate the diagnostic value of both the full and penalised model.

We know from previous research that probable FA to plant source foods dominates in adults,[3] but that both plant and animal source probable FA play important roles in school-age children.[2] Because the presentation of FA may depend on the type of food eliciting the reaction, patient history determinants of children with only plant source probable FA and of children with only animal source probable FA were compared univariably in an extra explorative analysis. The Lasso regression was then repeated in the source population of children with self-reported FA to plant source foods, and the outcome plant source probable FA was evaluated, as this concerned the largest group of children, and improved comparability to the adult population. Analyses were conducted with SPSS version 25 and R version 3.4.1.

Results

Adults

Of 862 adult subjects reporting symptoms to priority foods and completing phase II,[3] the 844 with available serology were evaluated in this study. Positive IgE serology matching the food reported to cause symptoms was identified in 207/844 (25%) subjects, who were classified as having probable FA. Table 1 shows the population characteristics of these subjects. Most adults had probable FA to hazelnut, followed by apple, peach, kiwi, carrot and walnut (Table S1). Complete data on the predictor variables, as required for multivariable analyses, was available for 807 subjects. There were no significant differences in demographics, allergic comorbidities, or reaction characteristics between the subjects with complete data (N=807) and the subjects with missing data on food serology or predictor variables (N=55), except that subjects from certain centres were more likely to have complete data (Table S2).

Univariable analysis revealed that shorter time until onset of the reaction, reproducibility of the reaction, and reporting of OAS, rhinoconjunctivitis or respiratory symptoms in response to the culprit food, were statistically significantly ($p<0.05$) associated with probable FA, as were allergic comorbidities AR and asthma, and demographic factors younger age and male sex (Table 1 and 2). Reporting of gastrointestinal (GI) symptoms and potential cardiovascular symptoms (i.e. fainting or dizziness) were associated with not having probable FA.

Table 1. Population characteristics

	Adults			Children		
	Probable FA (N = 207)	**No probable FA (N = 637)**	**p***	**Probable FA (N = 136)**	**No probable FA (N = 534)**	**p***
Age in years, *mean (±SD)*	35.9 (9.0)	37.8 (9.5)	0.012	9.0 (1.0)	8.9 (1.0)	0.138
Sex, *N (%)*						
Male	93 (44.9)	198 (31.1)	<0.001	68 (50.0)	254 (47.6)	0.612
Female	114 (55.1)	439 (68.9)		68 (50.0)	280 (52.4)	
Level of education[a], *N* (%)						
Low	69 (33.3)	208 (32.7)	0.856	82 (60.3)	297 (55.6)	0.326
High	138 (66.7)	429 (67.3)		54 (39.7)	237 (44.4)	
(Parental) smoking[b], *N* (%)	106 (51.2)	321 (50.4)	0.838	78 (57.4)	258 (48.4)	0.062
Allergic comorbidities, *N* (%)						
Allergic rhinitis	190 (91.8)	376 (59.0)	<0.001	101 (74.3)	234 (43.8)	<0.001
Asthma	81 (39.1)	119 (18.7)	<0.001	54 (39.7)	145 (27.2)	0.004
Atopic dermatitis	55 (26.6)	179 (28.1)	0.669	89 (65.4)	288 (53.9)	0.016
Reproducibility of reaction[c], *N*(%)	195 (95.6)	547 (88.1)	0.002	120 (90.9)	481 (92.3)	0.592
Time onset in minutes, *median (Q1-Q3)*	5.0 (1.0-15.0)	30.0 (10.0-120.0)	<0.001	5 (1.0-60.0)	60.0 (5.0-240.0)	<0.001
Symptoms[d], *N* (%)						
Oral allergy	169 (81.6)	214 (33.6)	<0.001	75 (55.1)	112 (21.0)	<0.001
Skin	79 (38.2)	259 (40.7)	0.524	108 (79.4)	345 (64.6)	0.001
Gastrointestinal	52 (25.1)	286 (44.9)	<0.001	38 (27.9)	205 (38.4)	0.024
Rhinoconjunctivitis	61 (29.5)	142 (22.3)	0.036	55 (40.4)	133 (24.9)	<0.001
Respiratory	44 (21.3)	71 (11.1)	<0.001	27 (19.9)	47 (8.8)	<0.001
Cardiovascular	15 (7.2)	79 (12.4)	0.041	6 (4.4)	18 (3.4)	0.560
Centre, *N* (%)						
Athens	5 (2.4)	17 (2.7)	<0.001	7 (5.1)	17 (3.2)	0.003
Lodz	26 (12.6)	88 (13.8)		30 (22.1)	174 (32.6)	
Madrid	19 (9.2)	63 (9.9)		22 (16.2)	43 (8.1)	
Reykjavik	21 (10.1)	189 (29.7)		26 (19.1)	128 (24.0)	
Utrecht	60 (29.0)	139 (21.8)		28 (20.6)	78 (14.6)	
Zurich	76 (36.7)	141 (22.1)		17 (12.5)	48 (9.0)	

[a]Low level of education: None, primary school only, secondary school only or lower level vocational training. High level of education: higher level vocational training, university or full-time student. For children, the highest level of education of the best educated parent was taken into account. [b]For adults: "Ever smoked for at least one year?" For children: "Has either parent smoked at least 1 cigarette (or alternative) a day since the birth of the child?" [c]Reproducibility of reaction: the reaction occurred more than once *(reference = once)*. [d]Oral allergy symptoms: itching/tingling/swelling of the mouth/lips/throat. Skin symptoms: rash/nettle sting/itchy skin. Rhinoconjunctivitis: runny/stuffy nose or red/sore/running eyes. Gastrointestinal symptoms: diarrhoea/vomiting. Respiratory symptoms: breathlessness. Cardiovascular symptoms: fainting or dizziness. *The p-values pertain to the comparison of the two preceding columns using the chi-square test, two-sample T-test or Mann-Whitney U test as appropriate for the variable's distribution.

Table 2. Associations of reaction characteristics, allergic comorbidities, and demographic factors with probable FA in subjects reporting food-related symptoms

	Adults				Children			
	Crude OR [i]	95%-CI	Adjusted OR[ii]	95%-CI	Crude OR[i]	95%-CI	Adjusted OR[ii]	95%-CI
Reaction characteristics								
Time until onset*	**0.94**	**0.90 - 0.96**	**0.96**	**0.93 - 0.99**	0.99	0.98 - 1.00	1.00	0.99 - 1.00
Reproducibility of reaction	**2.93**	**1.52 - 6.39**	**3.24**	**1.41 - 8.33**	0.83	0.44 - 1.70	0.75	0.34 - 1.72
Oral allergy symptoms	**8.79**	**6.02 - 13.12**	**5.62**	**3.61 - 8.93**	**4.63**	**3.12 - 6.91**	**3.43**	**2.07 - 5.71**
Skin symptoms	0.90	0.65 - 1.24	0.92	0.59 - 1.42	**2.11**	**1.36 - 3.37**	1.67	0.96 - 2.96
Gastrointestinal symptoms	**0.41**	**0.29 - 0.58**	**0.59**	**0.38 - 0.90**	**0.62**	**0.41 - 0.93**	0.86	0.52 - 1.42
Rhinoconjunctivitis	**1.46**	**1.02 - 2.07**	1.11	0.69 - 1.77	**2.05**	**1.38 - 3.03**	1.43	0.85 - 2.38
Respiratory symptoms	**2.15**	**1.41 - 3.25**	1.41	0.82 - 2.41	**2.57**	**1.52 - 4.28**	1.01	0.50 - 1.98
Cardiovascular symptoms	**0.55**	**0.30 - 0.95**	0.63	0.30 - 1.25	1.32	0.44 - 3.23	1.39	0.40 - 3.82
Allergic comorbidities								
Allergic rhinitis	**7.76**	**4.74 - 13.52**	**4.44**	**2.52 - 8.26**	**3.70**	**2.45 - 5.70**	**3.13**	**1.87 - 5.33**
Asthma	**2.80**	**1.98 - 3.94**	**1.88**	**1.21 - 2.95**	**1.77**	**1.19 - 2.61**	1.20	0.71 - 2.02
Atopic dermatitis	0.93	0.65 - 1.31	0.74	0.47 - 1.16	**1.62**	**1.10 - 2.41**	1.36	0.85 - 2.22
Demographic factors								
Age	0.98	0.96 - 1.00	0.98	0.96 - 1.00	1.16	0.95 - 1.42	0.99	0.78 - 1.26
Male sex	**1.81**	**1.31 - 2.49**	**2.34**	**1.55 - 3.57**	1.10	0.76 - 1.61	1.03	0.65 - 1.61
High level of education	0.97	0.70 - 1.36	1.01	0.64 - 1.59	0.83	0.56 - 1.21	0.93	0.57 - 1.50
(Parental) smoking	1.03	0.75 - 1.41	1.43	0.95 - 2.16	1.43	0.98 - 2.10	1.22	0.77 - 1.96
AUC			**0.86 (0.83 - 0.89)**				**0.76 (0.71 - 0.82)**	

[i] Unadjusted OR, results of univariable logistic regression analysis. [ii] Adjusted OR, results of multivariable logistic regression analysis with all covariates included, i.e. the full model. Model coefficients were adjusted for centre. *ORs for time until onset per 30 minutes. **Bold** indicates p<0.05. *OR, odds ratio; CI, confidence interval; AUC, area under the receiver operating characteristic curve.*

In multivariable analyses, the combination of all predictor variables resulted in an AUC of 0.86 (95%-CI 0.83-0.89), implying good discriminative ability. Seven patient history predictors (reaction time, reproducibility of the reaction, OAS, GI symptoms, asthma, AR, and sex) were found to independently and statistically significantly contribute to differentiation between presence and absence of probable FA (Table 2). The strongest of these predictors were reporting of OAS and AR comorbidity, with respective ORs of 5.62 (95%-CI 3.61-8.93) and 4.44 (95%-CI 2.52-8.26).

The results from Lasso regression are presented in Figure 1. The same seven patient history variables were selected to optimally predict probable FA (Figure 1), though the ORs were less extreme and the AUC of this parsimonious model (based on cross-validation) was lower (0.85 (95%-CI 0.82-0.87)), as expected.

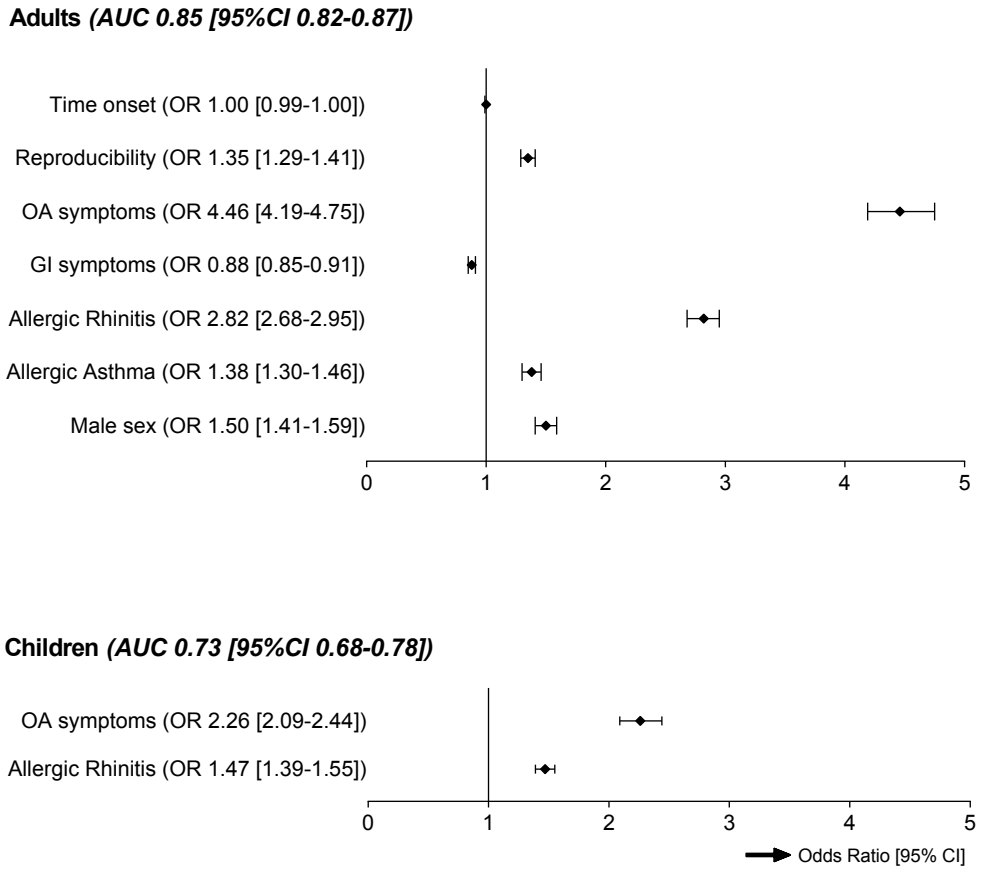

Figure 1. Independent predictors of probable FA in individuals reporting food-related symptoms, results from Lasso regression analysis. The 95% confidence intervals were calculated from standard errors obtained through 1000 bootstrap samples. *OA, oral allergy; GI, gastrointestinal.*

Children

As regards the population of school-age children, 702 subjects with self-reported FA completed phase II,[2] and 670 underwent food serology testing. A total of 136/670 (20%) children were found to have probable FA (Table 1). Children were mostly allergic to cow's milk, hen's egg, hazelnut, peanut, apple, kiwi, peach and walnut (Table S1). Multivariable analyses could be performed in 593 children with complete data on predictor variables. As seen in Table S2, the population characteristics of the children with complete data (N=593) were not significantly different to those of children with missing data on food serology or predictor variables (N=109).

Similarly to adults in univariable analyses, reporting of shorter time until onset of the reaction, OAS, rhinoconjunctivitis or respiratory symptoms, and having comorbid AR or asthma, were significantly associated with probable FA, and subjects reporting GI symptoms were less likely to have probable FA (Table 1 and 2). In contrast to adults, reporting skin symptoms and having comorbid atopic dermatitis, were positively and significantly associated with probable FA in children, and reproducibility of the reaction, although not statistically significant, was inversely associated with probable FA. None of the demographic factors age, sex, or level of education predicted probable FA in children.

All patient history variables combined in multivariable analysis, yielded a full model with an AUC of 0.76 (0.71-0.82). Two variables, which were also the strongest predictors in adults, were statistically significant for prediction of probable FA in children: OAS (OR 2.94 [95%-CI 1.75-4.97]) and AR comorbidity (OR 3.39 [95%-CI 1.98-5.91]) (Table 2). These two variables were also selected in the Lasso regression (Figure 1), which again resulted in less extreme ORs, and a lower cross-validation based AUC of 0.73 (95%-CI 0.68-0.78).

Plant *versus* animal source causative foods in children

Whereas the vast majority of adults with probable FA were allergic to plant source foods (188/207, 91%), probable FA in children was frequently caused by animal source foods (62/136, 46%) as well as by plant source foods (92/136, 68%) (Table S1). Table 3 shows that children with probable FA to only plant source foods (N=74) reported OAS and AR comorbidity more often than children with probable FA to only animal source foods (N=44). Furthermore, 58% of children with probable FA to animal source foods reported GI-symptoms, compared to only 13% of children with probable FA to plant source foods.

When probable FA to plant source foods rather than all priority foods was taken as the outcome of interest, in a source population of children with self-reported FA to plant source foods, Lasso regression selected OAS (OR 1.69, 95%-CI 1.57-1.82), rhinoconjunctivitis symptoms (OR 1.08, 95%-CI 1.04-1.13), GI-symptoms (OR 0.63,

95%-CI 0.59-0.66), and AR comorbidity (OR 3.10, 95%-CI 2.86-3.37), as contributive predictors. The cross-validation based AUC of this LASSO model focused on plant source probable FA, 0.81 (95%-CI 0.75-0.89), was more comparable to adults, than the AUC of the LASSO model for predicting probable FA due to any priority food.

Table 3. Population characteristics for plant *versus* animal source probable FA in children

	Plant source probable FA (N = 74)	**Animal source probable FA (N = 44)**	**p***
Age in years, *mean (±SD)*	9.2 (±1.0)	8.7 (±0.9)	0.017
Sex, *N (%)*			
Male	37 (50.0)	22 (50.0)	>0.99
Female	37 (50.0)	22 (50.0)	
Level of education parents, *N* (%)			
Low	49 (66.2)	26 (59.1)	0.437
High	25 (33.8)	18 (40.9)	
Parental smoking, *N* (%)	43 (58.1)	27 (61.4)	0.728
Allergic comorbidities, *N* (%)			
Allergic rhinitis	62 (83.8)	23 (52.3)	<0.001
Asthma	27 (36.5)	17 (38.6)	0.815
Atopic dermatitis	44 (59.5)	29 (65.9)	0.485
Reproducibility of reaction, *N* (%)	61 (87.1)	41 (93.2)	0.306
Time onset in minutes, *median (Q1-Q3)*	5.0 (1.0-120.0)	15.0 (3.0-90.0)	0.404
Symptoms, *N* (%)			
Oral allergy	45 (60.8)	15 (34.1)	0.005
Skin	60 (81.1)	32 (72.7)	0.290
Gastrointestinal	9 (12.2)	23 (52.3)	<0.001
Rhinoconjunctivitis	33 (44.6)	13 (29.5)	0.105
Respiratory	11 (14.9)	8 (18.2)	0.635
Cardiovascular	5 (6.8)	0 (0.0)	0.078
Centre, *N* (%)			0.002
Athens	3 (4.1)	1 (2.3)	
Lodz	16 (21.6)	11 (25.0)	
Madrid	13 (17.6)	6 (13.6)	
Reykjavik	7 (9.5)	16 (36.4)	
Utrecht	15 (20.3)	8 (18.2)	
Zurich	16 (31.6)	0 (0.0)	
Vilnius	4 (5.4)	2 (4.5)	

In 18/36 children, both animal and plant source foods caused probable FA (Table S1). These subjects are excluded in this table. *The p-values pertain to the comparison of the two preceding columns using the chi-square test, two-sample T-test or Mann-Whitney U test as appropriate for the variable's distribution. Exploratory analyses, not corrected for multiple testing.

Discussion

Experts describe patient history as the most important single test for diagnosing FA.[9] To our knowledge, the current study is the first to quantify the value of specific reported reaction characteristics (reaction time, reproducibility of reaction, symptoms) alongside allergic comorbidities and demographic factors, for predicting IgE sensitisation corresponding to the culprit food, making FA probable. We also found that combining seven independent predictors (reaction time, reproducibility of reaction, OAS, GI symptoms, AR comorbidity, asthma comorbidity, and sex) in a prediction model, allowed for good discrimination between presence and absence of probable FA in adults, with an AUC after cross-validation of 0.85. For school-age children, the most discriminative combination of predictors for probable FA was OAS and AR comorbidity, with a comparatively lower AUC of 0.73, but which tended to improve when focusing solely on plant source causative foods, the main source of FA in adults.

Based on expert opinion, current guidelines state that timing, reproducibility, symptoms and co-existing allergic diseases should be addressed in patient history for FA.[4, 5, 10] Our findings lend scientific evidence in support of these recommendations. A shorter time until onset of a reaction, reporting of OAS, rhinoconjunctivitis or respiratory symptoms upon ingestion of the culprit food, and AR or asthma comorbidity, were positively associated with probable FA in both adults and children. However, predictors of probable FA in children contrasted with those in adults in that time until onset and reproducibility of the reaction were not independently associated with probable FA in the paediatric multivariable analyses, and that skin symptoms tended to be more strongly associated with probable FA in children than in adults. An explanation for this could be that parents may not pick up on their child's reaction until later, when objective symptoms (i.e. skin symptoms) appear. Parents are also likely to ensure strict avoidance of a food after a child experiences a single adverse reaction, whereas adults may retry a food in case of mild symptoms, leading to reproducible reactions in adults and not in children. Another important difference between adults and children that affects which patient history determinants are associated with probable FA, is that adults are mainly allergic to plant source foods, whereas children are also likely to be allergic to animal source foods. Probable FAs to plant source foods rarely present with GI symptoms, whereas GI symptoms are often reported in relation to probable FA to animal source foods (Table 3). This observation explains why GI symptoms were inversely associated with probable FA in adults, but this association was only found in children when the analyses focused on plant source probable FA.

The strongest predictor of probable FA in both the adult and paediatric population, was reporting of OAS. Clinical experience teaches that this clearly identifiable

symptom is generally the first symptom that subjects with an IgE-mediated FA experience,[11, 12] though it is particularly associated with pollen-related FA. Pollen-related FA is a very common cause of FA in European adults and adolescents, and generally presents with mild OAS in reaction to raw fruits, vegetables and nuts that cross-react with pollen allergens to which the symptomatic individual is sensitised (most often PR-10 proteins found in birch, or profilin found in all pollen).[13, 14] The majority of subjects in our study were from birch endemic regions in Central and Northern Europe. In order to evaluate the relative importance of OAS independently of, and the modification of its predictive effect by, birch pollen sensitisation, we performed an additional analysis. We added IgE sensitisation to major birch pollen allergen Bet v 1,[13] and an interaction between OAS and IgE sensitisation to Bet v 1, to the full multivariable model. As expected, there was a statistically significant interaction between reporting of OAS and Bet v 1 sensitisation in adults ($p = 0.02$). OAS was particularly predictive of FA in those with Bet v 1 sensitisation (OR 11.8 [95%-CI 3.9-37.6]), and a less strong predictor in those without Bet v 1 sensitisation (OR 2.6 [95%-CI 1.4-4.9]). Nonetheless, OAS remained a statistically significant independent predictor of probable FA in adults. As birch pollen-related FA is not yet as common in 7- to 10-year-old children as in adults, it was not surprising that a similar interaction was not observed in children.

Although it goes beyond the scope of this paper to delve into geographical variation in the likelihood of probable FA, as this topic was extensively discussed in previous publications,[2, 3] it is worth noting that the effect of centre on probable FA in adults in multivariable analysis was strongest in countries known for high level of birch pollen sensitisation (Switzerland, Poland and The Netherlands). The effect of centre was no longer statistically significant after adjustment for Bet v 1 sensitisation in the full model. In Lasso regression analysis, only Switzerland, the EuroPrevall country with the most birch pollen sensitisation,[3] was selected as predictive of probable FA in adults. In the paediatric multivariable models, centre was not a statistically significant predictor in the full model with all covariates included, nor was it selected during Lasso regression. Apparently, most of the variation between centres in the paediatric population is explained by the other covariates included in the model (Table 2).

Previous studies taking the predictive value of patient history into account, tend to focus only on severity of reported symptoms. In a paediatric outpatient population selected for food challenge in Ireland and the UK, DunnGalvin *et al.* found that increasing severity of reported symptoms increased the likelihood of challenge-confirmed peanut, milk and egg allergy.[15] In a Dutch adult outpatient population, Klemans *et al.* observed no statistically significant association between reported symptom severity and challenge-confirmed peanut allergy.[16] For comparative purposes, we graded reported symptoms according to the severity classification used by DunnGalvin *et al.* in an additional analysis (Table S3).[15] Similarly to DunnGalvin *et*

al, the likelihood of probable FA tended to increase with increasing symptom severity in children in our study, though the trend was less clear in adults. Interestingly, our model with specific symptoms included independently (Table 2) rather than grouped in severity classifications (Table S3), was significantly better at discriminating between presence and absence of probable FA, specifically in adults (AUC = 0.86 [95%-CI 0.83-0.89) *versus* 0.81 [0.78-0.84], $p_{De\ Long's\ test} < 0.001$ in adults; and AUC 0.76 [0.71-0.82] *versus* 0.73 [0.68-0.79], $p_{De\ Long's\ test} = 0.43$ in children).

The high predictive ability of our multivariable models for probable FA in adults, which combine reaction time, reproducibility of reaction, OAS, GI symptoms, AR comorbidity, asthma comorbidity, and sex, may be useful in clinical practice. Our parsimonious model corrected for overfitting (Figure 1) aimed to be more generalisable to the general population of patients with food-related complaints (Table 2). Details for the prediction formula and accuracy measures corresponding to specific cutoffs of the formula's outcome score are available in Table S4 and S5. Of particular interest is the high negative predictive value of the prediction formula. If all adults with a prediction score smaller than 0.17 (= 46% of the population) were to be classified as not having probable FA, 95% of these adults would indeed not have a probable FA. This might be of interest to GPs for identifying adults in whom to conduct further IgE sensitisation testing, although formal validation of this formula should probably be performed before it can be used as such.

Although there were no food challenge outcomes available to assess the diagnostic value of patient history by comparing it to the reference standard for diagnosis of FA, our findings from prediction analyses yield essential evidence on the value of patient history in support of clinical practice. Our findings for probable FA are in line with expectations from expert opinion, according to which timing, reproducibility, symptoms and co-existing allergic diseases should be addressed in patient history for FA.[4, 5, 10] The individual weights provided for these patient history determinants of probable FA in the current study, may in the future inform physicians' decision-making in daily practice, i.e. to help avoid unnecessary IgE testing in adults reporting adverse reaction to (mainly plant source) foods. All in all, our findings reinforce the value of patient history in the diagnostic work-up of FA.

Acknowledgements

We would like to thank all the patients for their participation in the study, and Ewoud Schuit for critical revision of the manuscript. We acknowledge the support of the EuroPrevall project by the EU (grant FP6-FOOD-CT-2005-514000).

References

1. Burney PG, Potts J, Kummeling I, Mills EN, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitization in European adults. Allergy. 2014;69(3):365-71.
2. Lyons SA, Clausen M, Knulst AC, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, et al. Prevalence of Food Sensitization and Food Allergy in Children across Europe. J Allergy Clin Immunol Pract. 2020;8(8):2736-2746.e9.
3. Lyons SA, Burney PGJ, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, Clausen M, et al. Food Allergy in Adults: Substantial Variation in Prevalence and Causative Foods Across Europe. J Allergy Clin Immunol Pract. 2019;7(6):1920-8 e11.
4. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy. 2014;69(8):1008-25.
5. Skypala IJ, Venter C, Meyer R, deJong NW, Fox AT, Groetch M, et al. The development of a standardised diet history tool to support the diagnosis of food allergy. Clin Transl Allergy. 2015;5:7.
6. Kummeling I, Mills EN, Clausen M, Dubakiene R, Perez CF, Fernandez-Rivas M, et al. The EuroPrevall surveys on the prevalence of food allergies in children and adults: background and study methodology. Allergy. 2009;64(10):1493-7.
7. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. Eur Respir J. 1994;7(5):954-60.
8. Tibshirani R. Regression Shrinkage and Selection via the Lasso. J Roy Stat Soc B. 1996;58(1):267-88.
9. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol. 2018;141(1):41-58.
10. Sampson HA. Food allergy--accurately identifying clinical reactivity. Allergy. 2005;60 Suppl 79:19-24.
11. Turner PJ, Baumert JL, Beyer K, Boyle RJ, Chan CH, Clark AT, et al. Can we identify patients at risk of life-threatening allergic reactions to food? Allergy. 2016;71(9):1241-55.
12. Amlot PL, Kemeny DM, Zachary C, Parkes P, Lessof MH. Oral allergy syndrome (OAS): symptoms of IgE-mediated hypersensitivity to foods. Clin Allergy. 1987;17(1):33-42.
13. Werfel T, Asero R, Ballmer-Weber BK, Beyer K, Enrique E, Knulst AC, et al. Position paper of the EAACI: food allergy due to immunological cross-reactions with common inhalant allergens. Allergy. 2015;70(9):1079-90.
14. Lyons SA, Dijk AMV, Knulst AC, Alquati E, Le TM, Os-Medendorp HV. Dietary Interventions in Pollen-Related Food Allergy. Nutrients. 2018;10(10).
15. DunnGalvin A, Daly D, Cullinane C, Stenke E, Keeton D, Erlewyn-Lajeunesse M, et al. Highly accurate prediction of food challenge outcome using routinely available clinical data. J Allergy Clin Immunol. 2011;127(3):633-9 e1-3.
16. Klemans RJ, Broekman HC, Knol EF, Bruijnzeel-Koomen CA, Otten HG, Pasmans SG, et al. Ara h 2 is the best predictor for peanut allergy in adults. J Allergy Clin Immunol Pract. 2013;1(6):632-8 e1.

Supplemental files

Table S1. Types of foods causing probable food allergy

	Adults		Children	
	N	**%**	**N**	%
Any	207		136	
Animal source foods only	19	9.2	44	32.4
Plant source foods only	179	86.5	74	54.4
Both animal and plant source foods	9	4.3	18	13.2
Animal source foods				
Cow's milk	2	1.0	35	25.7
Hen's egg	8	3.9	26	19.1
Fish	7	3.4	8	5.9
Shrimp	18	8.7	9	6.6
Plant source foods				
Hazelnut	102	49.3	26	19.1
Walnut	25	12.1	14	10.3
Apple	81	39.1	22	16.2
Peach	70	33.8	16	11.8
Kiwi	34	16.4	19	14.0
Banana	7	3.4	12	8.8
Melon	14	6.8	1	0.7
Tomato	13	6.3	10	7.4
Carrot	30	14.5	10	7.4
Celery	8	3.9	8	5.9
Peanut	11	5.3	26	19.1
Soybean	2	1.0	6	4.4
Lentil	1	0.5	5	3.7
Wheat	9	4.3	5	3.7
Buckwheat	1	0.5	2	1.5
Corn	2	1.0	1	0.7
Sesame seed	0	0.0	2	1.5
Sunflower seed	2	1.0	3	2.2
Mustard seed	0	0.0	0	0.0
Poppy seed	0	0.0	0	0.0

Subjects could be allergic to more than one food. A total of 447 probable FAs were identified in 207 adults, and 266 probable FAs in 136 children.

Table S2. Comparison of subjects with complete data to those with missing data on food serology and predictors for multivariable analyses

	Adults			Children		
	Complete data (N = 807)	**Missing data (N=55)**	**p***	**Complete data (N = 593)**	**Missing data (N=109)**	**p***
Probable FA	201 (24.9)	6 (16.2)*	0.230	126 (21.2)	10 (13.0)*	0.090
Age in years, *mean (±SD)*	37.3 (±9.4)	37.3 (±10.1)	0.986	8.9 (±1.0)	8.9 (±0.9)	0.721
Sex, *N (%)*						
Male	276 (34.2)	20 (36.4)	0.744	283 (47.7)	52 (47.7)	0.997
Female	531 (65.8)	35 (63.6)		310 (52.3)	57 (52.3)	
Level of education[a], *N* (%)						
Low	267 (33.1)	17 (30.9)	0.740	338 (57.0)	55 (50.5)	0.206
High	540 (66.9)	38 (69.1)		255 (43.0)	54 (49.5)	
(Parental) smoking[b], *N* (%)	410 (50.8)	28 (50.9)	0.988	302 (50.9)	47 (43.5)	0.157
Allergic comorbidities, *N* (%)						
Allergic rhinitis	543 (67.3)	38 (69.1)	0.782	304 (51.3)	48 (44.0)	0.165
Asthma	191 (23.7)	13 (23.6)	0.996	184 (31.0)	26 (23.9)	0.133
Atopic dermatitis	229 (28.4)	10 (18.2)	0.102	342 (57.7)	58 (53.2)	0.387
Reproducibility of reaction[c], *N* (%)	728 (90.2)	31 (86.1)	0.422	545 (91.9)	86 (93.5)	0.602
Time onset in minutes, *median (Q1-Q3)*	20.0 (5.0-120.0)	10.0 (2.0-60.0)	0.069	30.0 (5.0-180.0)	12.5 (2.0-240.0)	0.264
Symptoms[d], *N*(%)						
Oral allergy	370 (45.8)	21 (38.2)	0.269	170 (28.7)	30 (27.5)	0.808
Skin	324 (40.1)	24 (43.6)	0.610	397 (66.9)	78 (71.6	0.344
Gastrointestinal	326 (40.4)	17 (30.9)	0.164	212 (35.8)	38 (34.9)	0.859
Rhinoconjunctivitis	194 (24.0)	13 (23.6)	0.946	171 (28.8)	26 (23.9)	0.287
Respiratory	110 (13.6)	8 (14.5)	0.849	69 (11.6)	8 (7.3)	0.187
Cardiovascular	370 (45.8)	4 (7.3)	0.505	24 (4.0)	1 (0.9)	0.156
Centre, *N* (%)						
Athens	22 (2.7)	1 (1.8)	<0.001	21 (3.5)	5 (4.6)	<0.001
Lodz	112 (13.9)	5 (9.1)		183 (30.9)	23 (21.1)	
Madrid	77 (9.5)	6 (10.9)		61 (10.3)	8 (7.3)	
Reykjavik	198 (24.5)	12 (21.8)		141 (23.8)	14 (12.8)	
Utrecht	197 (24.4)	2 (3.6)		101 (17.0)	16 (14.7)	
Zurich	201 (24.9)	29 (52.7)		56 (9.4)	15 (13.8)	

See legend table 1. Addition: probable FA could only be determined in subjects with available serology.

Table S3. Full logistic regression model with reaction severity rather than individual symptoms as covariates

	Adults				Children			
	Crude OR[i]	**95%-CI**	**Adjusted OR**[ii]	**95%-CI**	**Crude OR**[i]	**95%-CI**	**Adjusted OR**[ii]	**95%-CI**
Reaction characteristics								
Time until onset*	**0.94**	**0.90 - 0.96**	**0.95**	**0.92 - 0.97**	0.99	0.98 - 1.00	1.00	0.99 - 1.00
Reproducibility of reaction	**2.93**	**1.52 - 6.39**	**3.56**	**1.56 - 9.33**	0.83	0.44 - 1.70	0.64	0.30 - 1.44
Symptom severity[†]								
Grade I	*Reference*				*Reference*			
Grade II	**1.58**	**1.04 - 2.40**	1.37	0.84 - 2.24	**1.78**	**1.10 - 2.89**	**1.74**	**1.01 - 3.01**
Grade III	**2.73**	**1.79 - 4.19**	**2.54**	**1.50 - 4.32**	**2.83**	**1.70 - 4.71**	**2.19**	**1.21 - 3.96**
Grade IV	0.90	0.51 - 1.51	0.94	0.50 - 1.73	**3.19**	**1.54 - 6.42**	**2.95**	**1.31 - 6.55**
Allergic comorbidities								
Allergic rhinitis	**7.76**	**4.74 - 13.52**	**4.74**	**2.74 - 8.70**	**3.70**	**2.45 - 5.70**	**3.94**	**2.37 - 6.70**
Asthma	**2.80**	**1.98 - 3.94**	**1.92**	**1.24 - 2.98**	**1.77**	**1.19 - 2.61**	1.11	0.68 - 1.80
Atopic dermatitis	0.93	0.65 - 1.31	0.73	0.47 - 1.11	**1.62**	**1.10 - 2.41**	1.43	0.90 - 2.30
Demographic factors								
Age	0.98	0.96 - 1.00	0.98	0.96 - 1.00	1.16	0.95 - 1.42	1.00	0.80 - 1.27
Male sex	**1.81**	**1.31 - 2.49**	**2.18**	**1.47 - 3.25**	1.10	0.76 - 1.61	1.03	0.66 - 1.60
High level of education	0.97	0.70 - 1.36	0.91	0.59 - 1.41	0.83	0.56 - 121	0.90	0.55 - 1.45
(Parental) smoking	1.03	0.75 - 1.41	1.24	0.84 - 1.85	1.43	0.98 - 2.10	1.15	0.73 - 1.82
AUC			***0.81***				***0.73***	
			(0.78 - 0.84)				***(0.68 - 0.79)***	

*ORs for time until onset per 30 minutes. [†]Symptom severity was classified similarly to DunnGalvin *et al*: I. oral allergy or skin or gastrointestinal or rhinoconjunctivitis symptoms only; II. 2 systems; III. lower respiratory symptoms or 3 systems; IV. cardiovascular symptoms or 4 systems.[E15] [i]Unadjusted OR, results of univariable logistic regression analysis. [ii]Adjusted OR, results of multivariable logistic regression analysis with all covariates included, i.e. the full model. Model coefficients were adjusted for centre. **Bold** indicates p<0.05. *OR, odds ratio; CI, confidence interval; AUC, area under the receiver operating characteristic curve.*

Table S4. Combination of determinants for optimal prediction of probable FA in adults

	Beta	**OR**	**95%-CI**
Reaction characteristics			
Time until onset per 30 mins	-0.005	1.00	0.99-1.00
Reproducibility of reaction	0.30	1.35	1.29-1.41
Oral allergy symptoms *(OAS)*	1.49	4.46	4.19-4.75
Gastrointestinal symptoms *(GI)*	-0.13	0.88	0.85-0.91
Allergic comorbidities			
Allergic rhinitis *(AR)*	1.04	2.82	2.68-2.95
Asthma *(A)*	0.32	1.38	1.30-1.46
Demographic factors			
Male sex *(MS)*	0.40	1.50	1.41-1.59
Intercept	*-3.09*		

Above table shows the coefficients corresponding to the odds ratios (OR; =Exp(beta)) presented for adults in Figure 1. The coefficients from Lasso regression are used rather than those from the full logistic regression model, as the Lasso model is expected to be more generalisable. Regarding centre, only Zurich was found to have an effect on the likelihood of FA compared to other centres (beta = 0.20). The corresponding prediction score can be calculated as follows:

$1/(1+(e\text{^}\text{-}(\text{-}3.09 + (\text{Time}*\text{-}0.005) + (\text{Reproducibility}*0.30) + (\text{OAS}*1.49) + (\text{GI}*\text{-}0.13) + (\text{AR}*1.04) + (\text{A}*0.32) + \text{MS}*0.40) + (\text{Zurich}*0.20))))$.

Relevant information on accuracy and positivity thresholds is presented in Table S5.

Table S5. Accuracy measures for the combination of determinants for optimal prediction of probable FA in adults presented in Table S4

Accuracy measure optimised	Maximum value of accuracy measure	Cutoff on prediction score*		PFA	No PFA	Sensitivity	Specificity	PPV	NPV
-	-	0.50	<	119	557	41 (34-48)	92 (90-94)	63 (54-70)	82 (79-85)
			≥	82	49				
Sens	100%	0.05	<	0	83	**100 (98-100)**	14 (11-17)	28 (25-31)	100 (96-100)
			≥	201	523				
	95%	0.15	<	10	299	**95 (91-97)**	49 (45-53)	38 (34-43)	97 (94-98)
			≥	191	307				
Spec	100%	0.61	<	189	603	6 (3-10)	**100 (99-100)**	80 (55-93)	76 (72-78)
			≥	12	3				
	95%	0.52	<	134	576	33 (27-40)	**95 (93-97)**	69 (59-77)	81 (78-84)
			≥	67	30				
PPV	80%	0.54	<	157	595	22 (17-28)	98 (97-99)	**80 (68-88)**	79 (76-82)
			≥	44	11				
NPV	100%	0.05	<	0	83	100 (98-100)	14 (11-17)	28 (25-31)	**100 (96-100)**
			≥	201	523				
	95%	0.17	<	18	356	91 (86-94)	59 (55-63)	42 (38-47)	**95 (93-97)**
			≥	183	250				
Youden		0.24	<	39	452	**81 (75-86)**	**75 (71-78)**	51 (46-57)	92 (89-94)
			≥	162	154				
PPV + NPV		0.54	<	157	595	22 (17-28)	98 (97-99)	**80 (68-88)**	**79 (76-82)**
			≥	44	11				

*For prediction score, see Table S4. This table shows the sensitivity (sens), specificity (spec), positive predictive value (PPV), and negative predictive value (NPV) corresponding with a cutoff on the prediction score of 0.5 (i.e. risk of 50%), and alternative cutoffs on the prediction score corresponding with optimal sens, spec, PPV, NPV, Youden's index = maximum [sensitivity+specificity-1][E17], and maximum [PPV+NPV].

References supplemental files

E1. DunnGalvin A, Daly D, Cullinane C, Stenke E, Keeton D, Erlewyn-Lajeunesse M, et al. Highly accurate prediction of food challenge outcome using routinely available clinical data. J Allergy Clin Immunol. 2011;127(3):633-9 e1-3.
E2. Youden WJ. Index for rating diagnostic tests. Cancer. 1950;3(1):32-5.

Chapter 6

IgE to hazelnut allergen components does not predict hazelnut challenge outcome in Dutch adults

Manuscript submitted

Sarah A. Lyons, Paco M.J. Welsing, Mariam Hakobyan, Hannah M. Kansen, Edward F. Knol, Henny G. Otten, Ronald van Ree, André C. Knulst, Thuy-My Le

Abstract

Background
Component-resolved diagnostics (CRD) help predict hazelnut allergy (HA) in children, but are of unknown diagnostic value in adults. This study aimed to evaluate the diagnostic accuracy of IgE to hazelnut extract and components in adults.

Methods
A Dutch population of consecutively presenting adults suspected of HA, who underwent a double-blind placebo-controlled food challenge (DBPCFC), were included. Serum IgE to hazelnut extract and Cor a 1, 8, 9 and 14 was measured on ImmunoCAP. Diagnostic accuracy was assessed by area under the curve (AUC) analysis.

Results
Of 108 patients undergoing DBPCFC, 52 had challenge-confirmed HA: 20 based on objective and 32 based on subjective symptoms. At commonly applied cutoffs 0.1 and 0.35 kU_A/L, high sensitivity was observed for IgE to hazelnut extract and Cor a 1 (range 83-90%), and high specificity for IgE to Cor a 8, 9 and 14 (range 75-90%). However, the AUCs for hazelnut extract and components were too low for accurate prediction of HA (range 0.49-0.53). Combining hazelnut extract and component IgE measurements did not significantly improve accuracy. Higher IgE levels to Cor a 14 were tentatively associated with HA with objective symptoms, but the corresponding AUC still only reached 0.62.

Conclusions
Although hazelnut allergic adults are usually sensitised to hazelnut extract and Cor a 1, and hazelnut tolerant adults are usually not sensitised to Cor a 8, 9 or 14, neither IgE to hazelnut extract nor IgE to hazelnut components can accurately discriminate between presence and absence of HA in adults from a birch-endemic country.

Introduction

As hazelnut is the tree nut most commonly reported to cause food allergic reactions in European adults,[1-4] accurate diagnosis of hazelnut allergy (HA) is essential. Double-blind placebo-controlled food challenge (DBPCFC) is the reference standard for diagnosis. However, DBPCFC is resource-intensive, burdensome, and carries the risk of severe reactions. In addition, certain patients decline or are excluded from DBPCFC (e.g. patients a history of severe anaphylaxis, patient with chronic urticaria or pregnant women).[5, 6]

Other diagnostic tests in the evaluation of HA, which are less invasive and can be performed on anyone, include measurement of serum IgE levels to hazelnut extract, and more recently, hazelnut allergen components.[7, 8] Such serology tests, commonly referred to as component-resolved diagnostics (CRD), are readily available for hazelnut storage components Cor a 9 (11S globulin) and Cor a 14 (2S albumin), and hazelnut cross-reactive components Cor a 1 (pathogenesis related protein family 10 [PR-10] protein) and Cor a 8 (lipid transfer protein [LTP]). A recent systematic review and meta-analysis concludes that IgE to hazelnut extract, Cor a 9 and Cor a 14 can contribute to accurate identification of children with HA.[9] Some studies suggest that hazelnut CRD sensitisation profiles are also linked to specific clinical allergy phenotypes and may predict the risk of a severe reaction to hazelnut.[8, 10-12] Data on adults are scarce, and have been obtained from case-control studies[10, 13] or studies in mixed adult and paediatric populations[11, 14]. Findings based on an unselected fully adult population are not yet available.

The aim of this study was to evaluate the diagnostic performance of ImmunoCAP tests with hazelnut extract and components Cor a 1, Cor a 8, Cor a 9 and Cor a 14, individually and combined, for distinguishing between presence and absence of HA in adults. As has already been established for children, such data could reduce the need for DBPCFC for HA, and give hazelnut CRD a prominent place in food allergy diagnostic guidelines for adults.

Methods

Study population

All consecutive adult patients with suspected HA who underwent DBPCFC between August 2012 and January 2019 at the University Medical Centre Utrecht (UMCU), the Netherlands, were eligible for inclusion. Prior to DBPCFC, all patients were evaluated in the UMCU outpatient clinic. Patients with an inconclusive DBPCFC, or without leftover serum to determine missing IgE results, were excluded from analyses. The

study was approved by the research ethics committee of the UMCU (protocol number 18-428).

Data collection

Data on DBPFC results; serum IgE levels to hazelnut extract and hazelnut components Cor a 1, 8, 9 and 14; patient demographics (age, sex); and allergic comorbidities (asthma, atopic dermatitis, allergic rhinitis), were collected retrospectively from patients' medical files. IgE levels were determined using the ImmunoCAP platform (*Thermo Fisher Scientific Uppsala, Sweden*). In patients with missing IgE results, IgE levels were obtained using leftover serum stored in the department's serum bank and the UMCU's biobank. DBPCFC was performed according to international consensus protocols.[5, 6] During a 2-day approach in a hospital setting, hazelnut protein or placebo was administered orally in portions increasing every 20-30 minutes. A negative challenge was always followed by an open challenge test to confirm absence of symptoms. The outcomes of the challenges were discussed among local food allergy experts. The test was considered positive upon occurrence of objective symptoms, subjective symptoms in response to a minimum of three doses, or subjective symptoms lasting at least 45 minutes.[5, 6] Objective symptoms included urticaria, erythema, angioedema, conjunctivitis, rhinitis, vomiting, diarrhoea, cough, wheezing, stridor, hoarseness, objective dyspnoea, cyanosis, respiratory arrest, tachycardia, dysrhythmia, hypotension or cardiac arrest. Subjective symptoms included oral allergy symptoms, pruritus or pressure in the ear, local or generalised pruritus, subjective feeling of oral swelling, subjective eye symptoms (pruritus, irritation or burning of the eyes), sense of nasal congestion, nausea, abdominal pain, difficulty swallowing, subjective dyspnoea or dizziness. These criteria were agreed upon prior to data collection and statistical analysis.

Statistical analysis

Data on patient characteristics for those with HA *versus* those without HA, and for those with HA with objective symptoms *versus* those with HA with subjective symptoms or without HA, were presented in absolute number and percentage for categorical variables, and mean and standard deviation or median and interquartile range for continuous variables, and compared using the chi-square test, independent sample t-test or Mann-Whitney U test.

The diagnostic accuracy of IgE levels to hazelnut extract and each of the individual components was assessed by the area under the curve (AUC) of the receiver operating characteristic (ROC) and corresponding 95% confidence interval (CI). DeLong's test was used for statistical comparison of AUCs.[15] Sensitivity, specificity, positive and negative predictive values were obtained for cutoffs most commonly used in clinical practice: 0.1 and 0.35 kU_A/L. In case of sufficiently large

AUCs indicative of accurate discrimination, cutoffs for IgE levels corresponding to positive or negative predictive values >95% were to be determined.

To evaluate the diagnostic value of all the ImmunoCAP results combined (hazelnut extract, Cor a 1, 8, 9 and 14) for prediction of HA, multivariable logistic regression was applied. After determining the AUC of the full model including all ImmunoCAPs, Least Absolute Shrinkage and Selection Operator (Lasso) regression was used to determine the most discriminative combination of hazelnut extract and components. Lasso regression is a form of penalised regression, which selects only the most contributive predictors, and applies shrinkage of regression coefficients through cross-validation, to limit overfitting.[16] No multivariable analyses were performed for prediction of HA with objective symptoms because of the low number of patients with this outcome. Analyses were conducted with SPSS version 25 (IBM Corporation, Armonk, NY) and R version 3.4.1 (R Core Team, Vienna, Austria).

Results

Clinical characteristics

A total of 139 adults underwent hazelnut DBPCFC during the period of inclusion, of which 31 were excluded from analyses due to inconclusive DBPCFC (N=19) or a lack of serum for determining IgE levels (N=12). There were no statistically significant differences between included and excluded patients, except that included patients tended to be slightly younger (32 *versus* 38 years on average, Table S1).

Of the 108 included adults, 32 (30%) were male and 76 (70%) were female. A total of 52/108 (48%) were classified as hazelnut allergic, and 20/52 hazelnut allergic patients had objective symptoms during DBPCFC. Clinical characteristics of these patients are shown in Table 1. Atopic dermatitis and allergic rhinitis were significantly more common in hazelnut allergic than in hazelnut tolerant patients. There were no statistically significant differences in characteristics between the patients with objective symptoms and the patients with no symptoms or subjective symptoms in DBPCFC.

Of the subjects with complete data on hazelnut extract and all components (N=89/108), the most commonly occurring sensitisation pattern (IgE $\geq 0.35 kU_A/L$) comprised sensitisation to hazelnut extract and Cor a 1 (N=48/89, 54%, Table S2). Sensitisation to Cor a 8, 9 or 14 without co-existing sensitisation to Cor a 1 was detected in 4/89 subjects (4%, Table S2). Overall, 10 of the 89 challenged subjects with complete IgE data were not sensitised to hazelnut extract or any of the components (or 9 subjects based on an IgE cutoff of 0.1 Ku_A/L). Four of these subjects had HA according to DBPCFC, 3 with objective symptoms.

Table 1. Patient characteristics of adults with and without HA, and of adults with and without HA with objective symptoms

	Total (N=108)	HA (N=52)	No HA (N=56)	p*	Objective HA (N=20)	Subjective/ No HA (N=88)	p*
Age in years, *mean (±SD)*	32 (±13)	32 (±12)	33 (±14)	0.736	35 (±13)	32 (±13)	0.396
Male sex	32 (30)	11 (21)	21 (38)	0.063	3 (15)	29 (33)	0.112
Asthma	56 (52)	29 (56)	27 (48)	0.432	12 (60)	44 (50)	0.419
AD	58 (54)	33 (64)	25 (45)	0.050	11 (55)	47 (53)	0.898
AR	94 (87)	50 (96)	44 (79)	0.007	19 (95)	75 (85)	0.240

Values are expressed as N (%) unless otherwise specified. *Explorative analyses, no correction for multiple testing. *HA, hazelnut allergy; SD, standard deviation; AD, atopic dermatitis; AR, allergic rhinitis.*

Diagnostic accuracy of serology-based testing for HA

There were no significant differences in levels of IgE to hazelnut extract, Cor a 1, 8, 9 or 14 between patients with and without HA, nor between patients with objective symptoms and those with no symptoms or subjective symptoms (Table 1, Figure 1).

Subsequently, neither IgE to hazelnut extract nor IgE to individual hazelnut components was found to discriminate well between presence or absence of HA, with AUCs ranging from 0.49 to 0.53 (Figure 2A). The full multivariable logistic regression model containing all IgE variables (hazelnut extract, Cor a 1, 8, 9 and 14) had an AUC of 0.61, and the Lasso regression model, which selected all IgE variables as the optimal predictive combination, had an AUC of 0.58, but these AUC values were not significantly larger than those of any of the individual serology tests ($P_{De\ Long's\ test} > 0.05$). Because of the low AUC values, no cutoffs with optimum positive or negative predictive values were explored.

Table 2 reveals high sensitivity of hazelnut extract and Cor a 1 (range 83-90%) and high specificity of Cor a 8, 9 and 14 (range 75-90%) for HA when considering commonly used cutoffs (0.1 or 0.35 kU_A/L). In clinical practice, this means that hazelnut allergic adults are likely to be sensitised to hazelnut extract and Cor a 1, and hazelnut tolerant adults are unlikely to be sensitised to Cor a 8, 9 and 14. The positive and negative predictive values of hazelnut extract and components were low, and approximately corresponded with the prevalence of HA (52/108, 48%) and hazelnut tolerance (56/108, 52%) in the study population (no matter the cutoff), as expected based on the finding that IgE levels to hazelnut extract and components had limited association with HA (Table 1, Figure 2A).

Diagnostic accuracy of serology-based testing for HA with objective symptoms

IgE to Cor a 14 showed a tendency towards association with objective symptoms (p=0.08), but the corresponding AUC was still low at 0.62 and not significantly larger than the AUC of the other serology tests, which ranged from 0.53 to 0.59 (Figure

2B). Regarding sensitivity, specificity, and positive predictive value, the observations for HA with objective symptoms *versus* HA with subjective symptoms/no HA, were similar to those for presence *versus* absence of HA (Table 3). The highest sensitivity was observed for hazelnut extract and Cor a 1 (79-85%), the highest specificity for Cor a 8, 9 and 14 (74-88%), and positive predictive values of all IgE measurements were low. Although negative predictive values appeared higher (75-86%), they approximately corresponded to the prevalence (and therefore a priori probability) of no HA or HA with subjective symptoms in our study population (88/108, 81%) indicating no added diagnostic value.

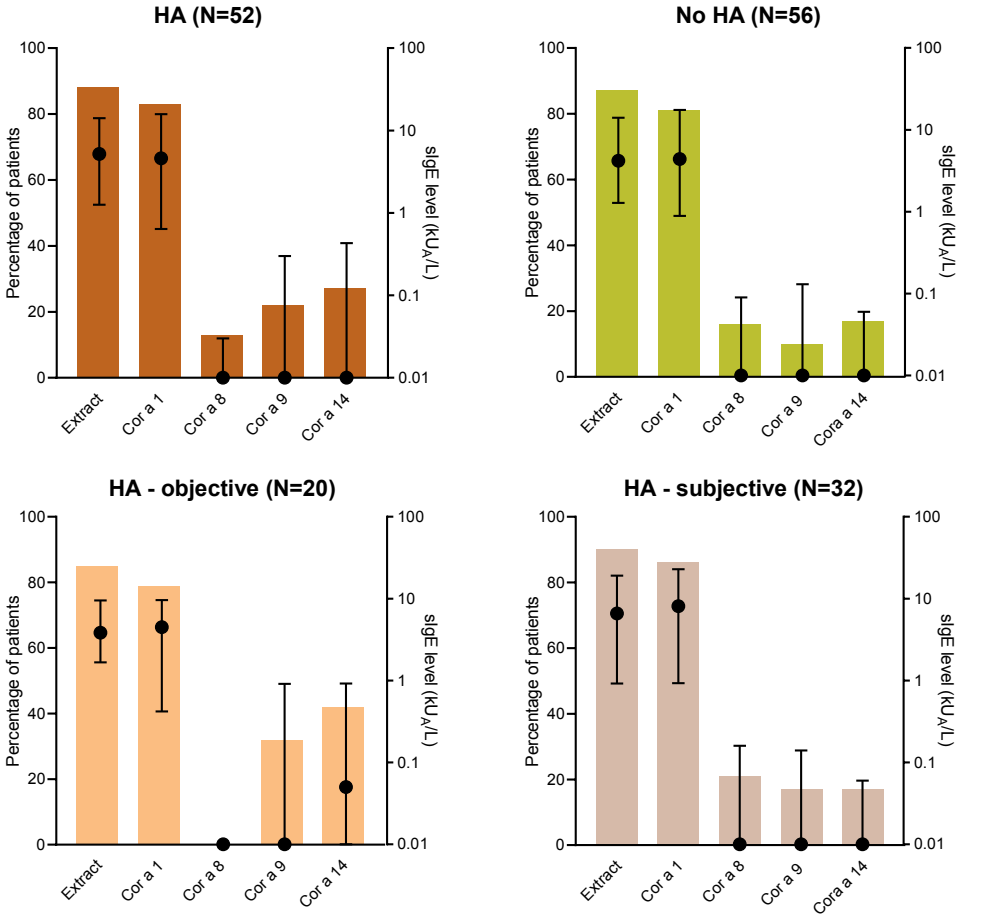

Figure 1. Percentage of patients with sensitisation to hazelnut extract or components and corresponding IgE levels, stratified to DBPCFC outcome

Of the in total 108 patients, serology results for hazelnut extract were available in 101, for Cor a 1 in 100, for Cor a 8 in 90, for Cor a 9 in 100, and for Cor a 14 in 101 patients. Sensitisation was considered present if IgE ≥ 0.35kU$_A$/L. For IgE level, medians and interquartile ranges are displayed on a logarithmic scale (base 10). DBPCFC, double-blind placebo-controlled food challenge.

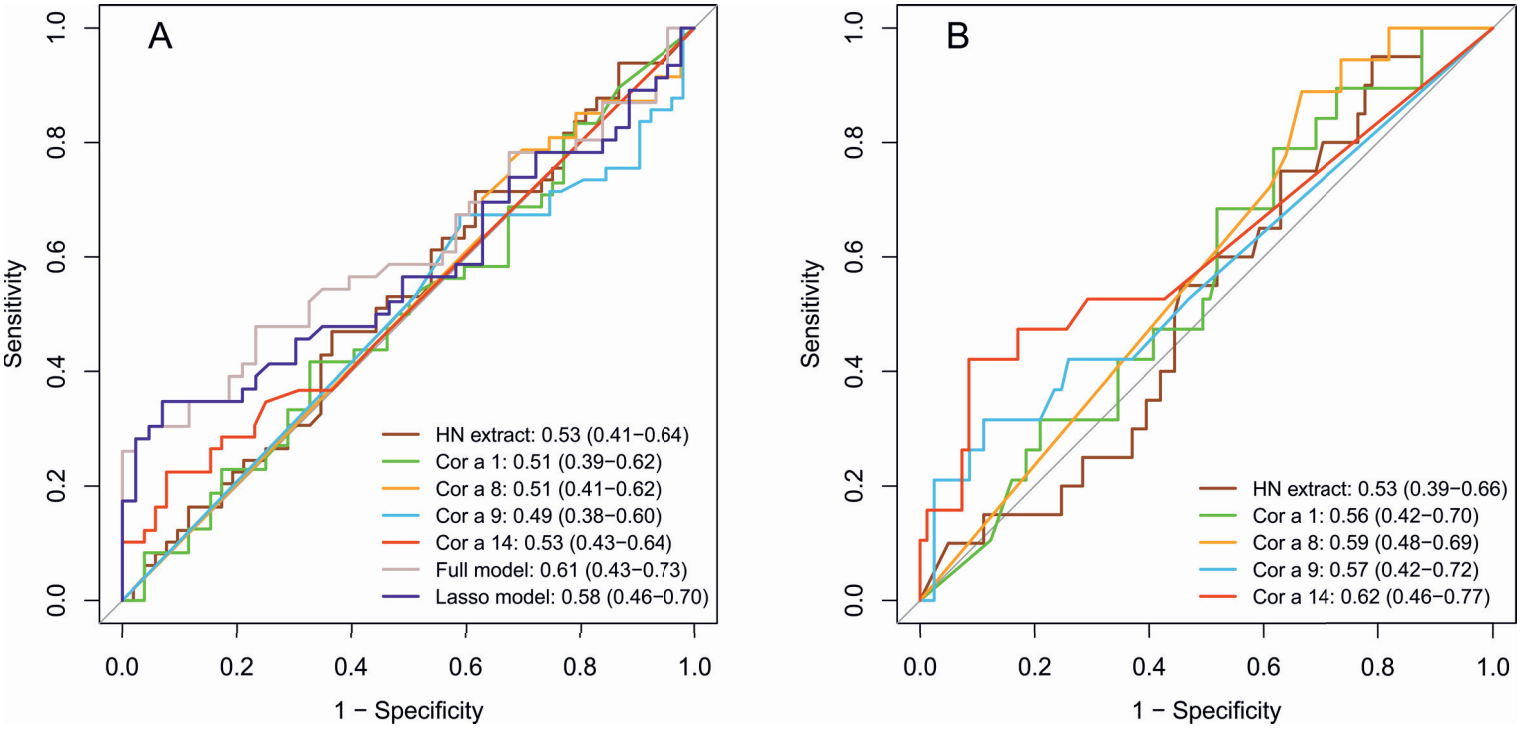

Figure 2. ROC-curves of serology tests for predicting presence of allergy (A) and objective symptoms (B) during DBPCFC
The area under the curve (95% confidence interval) is presented in the plot legends. In figure A, the full model contains IgE to Hazelnut extract, Cor a 1, Cor a 8, Cor a 9 and Cor a 14. All IgE variables were also selected in the LASSO regression model. No multivariable models were developed for outcome B due to the small number of subjects with the outcome of interest (N=20 with objective symptoms). *ROC, receiver operating characteristic; DBPCFC, double-blind placebo-controlled food challenge.*

Table 2. Measures of diagnostic accuracy for HA of hazelnut extract and components at cutoffs applied in daily practice

sIgE to:	N	Cutoff (kU_A/L)		HA	No HA	PPV	NPV	Sensitivity	Specificity
Hazelnut extract	101	0.10	≥	44	45	49.4 [39.3-59.6]	58.3 [32.0-80.7]	89.8 [78.2-95.6]	13.5 [6.7-25.3]
			<	5	7				
		0.35	≥	43	45	48.9 [38.7-59.1]	53.8 [29.1-76.8]	87.8 [75.8-94.3]	13.5 [6.7-25.3]
			<	6	7				
Cor a 1	100	0.10	≥	40	42	48.8 [38.3-59.4]	55.6 [33.7-74.5]	83.3 [70.4-91.3]	19.2 [10.8-31.9]
			<	8	10				
		0.35	≥	40	42	48.8 [38.3-59.4]	55.6 [33.7-74.5]	83.3 [70.4-91.3]	19.2 [10.8-31.9]
			<	8	10				
Cor a 8	90	0.10	≥	9	10	44.4 [24.6-66.3]	46.5 [35.4-58.0]	17.4 [9.1-30.7]	76.7 [62.3-86.8]
			<	38	33				
		0.35	≥	6	7	46.2 [23.2-70.9]	46.8 [36.0-57.8]	12.8 [6.0-25.2]	83.7 [70.0-91.9]
			<	41	36				
Cor a 9	100	0.10	≥	16	13	55.2 [37.5-71.6]	53.5 [42.0-64.6]	32.7 [21.2-46.6]	74.5 [61.1-84.5]
			<	33	38				
		0.35	≥	11	5	68.8 [44.4-85.8]	54.8 [44.1-65.0]	22.4 [13.0-35.9]	90.2 [79.0-95.7]
			<	38	46				
Cor a 14	101	0.10	≥	14	11	56.0 [37.1-73.3]	53.9 [42.8-64.7]	28.6 [17.8-42.4]	78.8 [66.0-87.8]
			<	35	41				
		0.35	≥	13	9	59.1 [38.7-76.7]	54.4 [43.5-65.0]	26.5 [16.2-40.3]	82.7 [70.3-90.6]
			<	36	43				

The positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity are presented (% [95% confidence interval]) for each of the investigated serology tests at cutoffs most commonly used in daily practice: 0.1 kU/L and 0.35 kU/L. N, number of patients included; HA, hazelnut allergy.

Table 3. Measures of diagnostic accuracy for objective HA of hazelnut extract and components at cutoffs applied in daily practice

sIgE to:	N	Cutoff (kU_A/L)		HA	No HA	PPV	NPV	Sensitivity	Specificity
Hazelnut extract	101	0.10	≥	17	72	19.1 [12.3-28.5]	75.0 [46.8-91.1]	85.0 [64.0-94.8]	11.1 [6.0-19.8]
			<	3	9				
		0.35	≥	17	71	19.3 [12.4-28.8]	76.9 [49.7-91.8]	85.0 [64.0-94.8]	12.3 [6.8-21.3]
			<	3	10				
Cor a 1	100	0.10	≥	15	67	18.3 [11.4-28.0]	77.8 [54.8-91.0]	78.9 [56.7-91.5]	17.3 [10.6-26.9]
			<	4	14				
		0.35	≥	15	67	18.3 [11.4-28.0]	77.8 [54.8-91.0]	78.9 [56.7-91.5]	17.3 [10.6-26.9]
			<	4	14				
Cor a 8	90	0.10	≥	1	18	5.3 [0.9-24.6]	76.1 [65.0-84.5]	5.6 [1.0-25.8]	75.0 [63.9-83.6]
			<	17	54				
		0.35	≥	0	13	0.0 [0.0-22.8]	76.6 [66.0-84.7]	0.0 [0.0-17.6]	81.9 [71.5-89.1]
			<	18	59				
Cor a 9	100	0.10	≥	8	21	27.6 [14.7-45.7]	84.5 [74.3-91.1]	42.1 [23.1-63.7]	74.1 [63.6-82.4]
			<	11	60				
		0.35	≥	6	10	37.5 [18.5-61.4]	84.5 [75.3-90.7]	31.6 [15.4-54.0]	87.7 [78.7-93.2]
			<	13	71				
Cor a 14	101	0.10	≥	9	16	36.0 [20.2-55.5]	86.8 [77.4-92.7]	47.4 [27.3-68.3]	80.5 [70.6-87.6]
			<	10	66				
		0.35	≥	8	14	36.4 [19.7-57.0]	86.1 [76.8-92.0]	42.1 [23.1-63.7]	82.9 [73.4-89.5]
			<	11	68				

The positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity are presented (% [95% confidence interval]) for each of the investigated serology tests at cutoffs most commonly used in daily practice: 0.1 kU/L and 0.35 kU/L. N, number of patients included; HA, hazelnut allergy.

Discussion

Testing for IgE sensitisation to hazelnut extract, and increasingly often for IgE sensitisation to hazelnut allergen components, is standard practice in the diagnostic work-up of HA in adults. However, according to the current study, neither IgE to hazelnut extract nor IgE to hazelnut components Cor a 1, 8, 9 or 14 can accurately predict hazelnut challenge outcomes in Dutch adults with suspected HA.

Findings for Cor a 9 and Cor a 14 in adults contrast with findings in children

Although IgE levels to hazelnut storage proteins Cor a 9 and Cor a 14 in our data tended to reach higher values in hazelnut allergic than in hazelnut tolerant adults (Table 1, Figure 1A), and in adults with objective symptoms than in adults with no or subjective symptoms to hazelnut (Table 2, Figure 1B), the differences were mostly negligible, and the corresponding AUCs were low. This appears to contrast with literature on children, according to which Cor a 9 and 14 in particular, are associated with HA.[9, 12, 17-21] Regarding prediction of HA in adults, no previous study has, to our knowledge, reported AUC values for hazelnut extract or components for discriminating between presence and absence of HA in an unselected population. However, in agreement with our findings, Hansen *et al.* found no difference in levels of IgE to Cor a 9 between hazelnut allergic adults and hazelnut tolerant pollen-allergic controls from Denmark, Switzerland and Spain.[13] Regarding prediction of HA with objective symptoms in adults, Masthoff *et al.* obtained AUC values of 0.66 and 0.67 for Cor a 9 and 14 respectively, and Datema *et al.* found AUCs of 0.70 and 0.71, which were both slightly higher than our respective AUC estimates of 0.57 and 0.62 (Figure 1B).[10, 11] These discrepancies are potentially explained by Masthoff's case-control approach and Datema's inclusion of children as well as adults. In comparison, AUC values of Cor a 9 and 14 for entirely paediatric populations from similar parts of Europe as the adults in the current study, are much higher: up to 0.80 for Cor a 9 and 0.89 for Cor a 14 for prediction of HA,[17, 20] and 0.87 for Cor a 9 and 0.80 for Cor a 14 for prediction of HA with objective symptoms.[10]

Interpretation of AUC values

The accuracy of a test as measured by AUC is a tradeoff between sensitivity and specificity,[22] as was also observed in the results of this study. At cutoffs frequently applied in clinical practice (0.1 and 0.35 kU_A/L), Dutch hazelnut allergic adults are mostly sensitised to hazelnut extract and to Cor a 1 (high sensitivity), but so are hazelnut tolerant adults (low specificity). On the other hand, hazelnut tolerant adults are generally not sensitised to Cor a 9 or 14 (high specificity), but neither are the majority of hazelnut allergic adults (low sensitivity). Although AUC values were not always available, similar patterns of high sensitivity (but low specificity) of hazelnut extract and Cor a 1, and high specificity (but low sensitivity) of Cor a 9 and 14, were

observed for prediction of HA or HA with objective symptoms in previously published data in predominantly adult populations from Europe.[10, 11, 13, 14]

Cor a 1 sensitisation affects the diagnostic value of hazelnut CRD in adults

Patterns of hazelnut component sensitisation in paediatric populations differ considerably from those in adults, particularly in that the majority of hazelnut allergic children are sensitised to Cor a 9 or 14, but not to Cor a 1.[9] Eighty-two percent of adults in the current study were sensitised to Cor a 1 (IgE ≥0.35 kU_A/L). Cor a 1 sensitisation occurs as a result of cross-reactivity with major birch pollen allergen Bet v 1, and likely affects the diagnostic value of CRD in several ways. First of all, Cor a 1 sensitisation itself is poorly associated with hazelnut challenge outcome, because symptoms in subjects with so-called birch pollen-related HA are generally mild or subjective and therefore difficult to interpret, and often depend on the degree of (heat) processing and sometimes on season.[23-25] Secondly, the majority of hazelnut allergic adults in this study (54%) had isolated Cor a 1 sensitisation, leading to a much lower sensitivity (and inherently AUC) of Cor a 9 and/or 14. Furthermore, in those subjects with polysensitisation to hazelnut components, we do not know which component is responsible for symptoms during DBPCFC. We did investigate the independent association of each component with HA by including all components as covariates in multivariable analysis, but the power to explore interaction between the different components was lacking. It would be interesting to repeat our research in an even larger population of adults from birch-endemic regions, so as to have more subjects with monosensitisation to Cor a 9 and 14 for study, and perhaps to explore if the ratio between IgE level to hazelnut storage proteins and Cor a 1 or birch affects prediction of hazelnut challenge outcome. This would also provide the opportunity to explore the hypothesis that sensitisation to birch and related PR-10 proteins may in some way inhibit (the clinical presentation of) sensitisation to other plant food allergens, such as storage proteins and LTP.[11, 13, 26]

IgG antibodies may affect the diagnostic value of hazelnut CRD in adults

One also ought to realise that ImmunoCAP quantifies allergen-specific IgE levels, but does not take presence of allergen-specific IgG antibodies into account.[27] IgG against food allergens indicates repeated exposure.[28] It is therefore conceivable that food-allergen specific IgG levels may be higher in adults than in children. Food allergen-specific IgG antibodies, particularly IgG_4 antibodies, have the potential to counteract symptom induction through IgE.[24, 29] If Cor a 9 or Cor a 14 specific IgG antibodies block an IgE-induced allergic response in some (but not all) adults with IgE sensitisation to Cor a 9 or 14, this phenomenon may also play a role the finding that IgE levels to Cor a 9 or 14 do not predict hazelnut allergy in adults, in contrast to children. Although the necessary data were lacking to explore this hypothesis in current study, further insight could be gained in future studies by assessing allergen-specific IgE/IgG_4 ratios.[30]

Findings on 2S albumins in HA contrast with findings on 2S albumins in peanut
Another interesting observation deserving attention because of contrast with our findings regarding HA, is that IgE to 2S albumins is strongly associated with peanut allergy in Dutch adults, even to the degree that cutoffs for Ara h 2 and 6 with 100% positive predictive values could be obtained.[31, 32] On one hand, this could be because a much larger proportion of peanut allergic than hazelnut allergic adults is sensitised to 2S albumins, and IgE to peanut PR-10 protein Ara h 8 is less clinically relevant for peanut allergy than Cor a 1 for HA. Alternatively, IgE to 2S albumins may be less clinically relevant for HA than for peanut allergy, for example if Cor a 14 sensitisation were due to cross-reactivity with 2S albumins in other food sources to which the patient is actually allergic. Cross-reactivity between Cor a 14 and Ara h 2 is low,[33] but between Cor a 14 and walnut 2s albumin Jug r 1 is high.[34] Perhaps the Cor a 14 sensitised individual is really walnut allergic.

Strengths and limitations
A limitation of the current study was the retrospective data collection, and the necessary selection of patients with conclusive DBPCFC and available serology results. However, the comparability of included and excluded patients (Table S1) make it unlikely that this selection resulted in bias. Furthermore, considering the small number of patients with objective symptoms in our study population, it is important to realise that our analyses with regard to severity of HA were merely explorative and should be interpreted as such. We also acknowledge that IgE to minor hazelnut allergens, such as 7S globulin Cor a 11, oleosins Cor a 12 and 13, or profilin Cor a 2, was not measured in the current study, but may be present in some patients.[35] The clinical relevance of these allergens in adults is presently unclear,[35] and would be an interesting topic for future exploration, especially as 4 subjects without sensitisation to hazelnut extract or components in our study had positive DBPCFC, 3 with objective symptoms.

Nonetheless, this study investigated all commercially available ImmunoCAP tests for hazelnut components in a large sample of consecutively presenting adults, who all underwent standardised double-blind placebo-controlled hazelnut challenge. We demonstrate that, although hazelnut allergic adults were generally sensitised to hazelnut extract and Cor a 1, and hazelnut tolerant adults were generally not sensitised Cor a 8, 9 or 14, neither IgE to hazelnut extract nor IgE to hazelnut components can accurately discriminate between presence and absence of HA in adult individuals with suspected HA from birch-endemic regions. Where some studies have been able to present cutoff levels of IgE with optimal positive or negative predictive values for food allergies and therefore the ability to reduce the need for DBPCFC,[31, 32, 36] the current findings indicate that such IgE cutoffs cannot be determined for HA in adults from birch-endemic regions. Some previous studies suggest exclusion of pollen-allergic subjects to gain true insight into the importance

of storage protein sensitisation in hazelnut allergic adults,[3, 13] but the clinical implications of such a study in birch-endemic Europe would be limited due to the fact that the vast majority of presenting patients are, in fact, allergic to birch pollen. For now, DBPCFC is required to diagnose (severity of) hazelnut allergy in adults in birch territory, though future studies increasing the sample size to include more subjects with Cor a 9 or 14 monosensitisation or taking the blocking potential of IgG antibodies into account, could expand our knowledge on the diagnostic value of hazelnut CRD in adults. Furthermore, it is worth acknowledging that alternative and upcoming diagnostic modalities, such as the basophil activation test (BAT), may be of particular interest in the study population at hand. The BAT is reported to be potentially useful for assessing clinical relevance of sensitisation to PR-10 proteins, and could help identify whether Cor a 1 sensitisation accounts for a hazelnut allergic reaction.[30, 37-39]

Conclusions

In conclusion, IgE to currently known and commercially available hazelnut allergen components does not accurately predict HA in adults from birch-endemic regions, and DBPCFC currently remains the tool of choice for final diagnosis of HA in this particular population.

Acknowledgements

We thank L. Bok and A. Kooij of the Centre of Translational Immunology (University Medical Centre Utrecht, the Netherlands) for performing ImmunoCAP measurements. ImmunoCAP material was kindly provided by Thermo Fisher Scientific (Uppsala, Sweden).

References

1. Lyons SA, Burney PGJ, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, Clausen M, et al. Food Allergy in Adults: Substantial Variation in Prevalence and Causative Foods Across Europe. J Allergy Clin Immunol Pract. 2019;7(6):1920-8 e11.
2. Woods RK, Abramson M, Bailey M, Walters EH. International prevalences of reported food allergies and intolerances. Comparisons arising from the European Community Respiratory Health Survey (ECRHS) 1991-1994. Eur J Clin Nutr. 2001;55(4):298-304.
3. McWilliam VL, Perrett KP, Dang T, Peters RL. Prevalence and natural history of tree nut allergy. Ann Allergy Asthma Immunol. 2020;124(5):466-472.
4. McWilliam V, Koplin J, Lodge C, Tang M, Dharmage S, Allen K. The Prevalence of Tree Nut Allergy: A Systematic Review. Curr Allergy Asthma Rep. 2015;15(9):54.
5. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. Standardization of food challenges in patients with immediate reactions to foods - position paper from the European Academy of Allergology and Clinical Immunology. Allergy. 2004;59(7):690-7.
6. Taylor SL, Hefle SL, Bindslev-Jensen C, Atkins FM, Andre C, Bruijnzeel-Koomen C, et al. A consensus protocol for the determination of the threshold doses for allergenic foods: how much is too much? Clin Exp Allergy. 2004;34(5):689-95.
7. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy. 2014;69(8):1008-25.
8. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, et al. EAACI Molecular Allergology User's Guide. Pediatr Allergy Immunol. 2016;27 Suppl 23:1-250.
9. Nilsson C, Berthold M, Mascialino B, Orme M, Sjolander S, Hamilton R. Allergen components in diagnosing childhood hazelnut allergy: Systematic literature review and meta-analysis. Pediatr Allergy Immunol. 2020;31(2):186-96.
10. Masthoff LJ, Mattsson L, Zuidmeer-Jongejan L, Lidholm J, Andersson K, Akkerdaas JH, et al. Sensitisation to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. J Allergy Clin Immunol. 2013;132(2):393-9.
11. Datema MR, van Ree R, Asero R, Barreales L, Belohlavkova S, de Blay F, et al. Component-resolved diagnosis and beyond: Multivariable regression models to predict severity of hazelnut allergy. Allergy. 2018;73(3):549-59.
12. Faber MA, De Graag M, Van Der Heijden C, Sabato V, Hagendorens MM, Bridts CH, et al. Cor a 14: missing link in the molecular diagnosis of hazelnut allergy? Int Arch Allergy Immunol. 2014;164(3):200-6.
13. Hansen KS, Ballmer-Weber BK, Sastre J, Lidholm J, Andersson K, Oberhofer H, et al. Component-resolved in vitro diagnosis of hazelnut allergy in Europe. J Allergy Clin Immunol. 2009;123(5):1134-41, 41 e1-3.
14. Datema MR, Zuidmeer-Jongejan L, Asero R, Barreales L, Belohlavkova S, de Blay F, et al. Hazelnut allergy across Europe dissected molecularly: A EuroPrevall outpatient clinic survey. J Allergy Clin Immunol. 2015;136(2):382-91.
15. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988;44(3):837-45.
16. Tibshirani R. Regression Shrinkage and Selection via the Lasso. J Roy Stat Soc B. 1996;58(1):267-88.
17. Beyer K, Grabenhenrich L, Hartl M, Beder A, Kalb B, Ziegert M, et al. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. Allergy. 2015;70(1):90-8.

18. Brandstrom J, Nopp A, Johansson SG, Lilja G, Sundqvist AC, Borres MP, et al. Basophil allergen threshold sensitivity and component-resolved diagnostics improve hazelnut allergy diagnosis. Clin Exp Allergy. 2015;45(9):1412-8.
19. Buyuktiryaki B, Cavkaytar O, Sahiner UM, Yilmaz EA, Yavuz ST, Soyer O, et al. Cor a 14, Hazelnut-Specific IgE, and SPT as a Reliable Tool in Hazelnut Allergy Diagnosis in Eastern Mediterranean Children. J Allergy Clin Immunol Pract. 2016;4(2):265-72 e3.
20. Eller E, Mortz CG, Bindslev-Jensen C. Cor a 14 is the superior serological marker for hazelnut allergy in children, independent of concomitant peanut allergy. Allergy. 2016;71(4):556-62.
21. Carraro S, Berardi M, Bozzetto S, Baraldi E, Zanconato S. COR a 14-specific IgE predicts symptomatic hazelnut allergy in children. Pediatr Allergy Immunol. 2016;27(3):322-4.
22. Yin J, Vogel RL. Using the roc curve to measure association and evaluate prediction accuracy for a binary outcome. . Biom Biostat Int J. 2017;5(3):91-103.
23. Werfel T, Asero R, Ballmer-Weber BK, Beyer K, Enrique E, Knulst AC, et al. Position paper of the EAACI: food allergy due to immunological cross-reactions with common inhalant allergens. Allergy. 2015;70(9):1079-90.
24. Geroldinger-Simic M, Zelniker T, Aberer W, Ebner C, Egger C, Greiderer A, et al. Birch pollen-related food allergy: clinical aspects and the role of allergen-specific IgE and IgG4 antibodies. J Allergy Clin Immunol. 2011;127(3):616-22 e1.
25. Lyons SA, Dijk AMV, Knulst AC, Alquati E, Le TM, Os-Medendorp HV. Dietary Interventions in Pollen-Related Food Allergy. Nutrients. 2018;10(10).
26. Fernandez-Rivas M, Bolhaar S, Gonzalez-Mancebo E, Asero R, van Leeuwen A, Bohle B, et al. Apple allergy across Europe: how allergen sensitisation profiles determine the clinical expression of allergies to plant foods. J Allergy Clin Immunol. 2006;118(2):481-8.
27. van Hage M, Hamsten C, Valenta R. ImmunoCAP assays: Pros and cons in allergology. J Allergy Clin Immunol. 2017;140(4):974-7.
28. Stapel SO, Asero R, Ballmer-Weber BK, Knol EF, Strobel S, Vieths S, et al. Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI Task Force Report. Allergy. 2008;63(7):793-6.
29. James LK, Till SJ. Potential Mechanisms for IgG4 Inhibition of Immediate Hypersensitivity Reactions. Curr Allergy Asthma Rep. 2016;16(3):23.
30. Santos AF, Brough HA. Making the Most of In Vitro Tests to Diagnose Food Allergy. J Allergy Clin Immunol Pract. 2017;5(2):237-48.
31. Klemans RJ, Broekman HC, Knol EF, Bruijnzeel-Koomen CA, Otten HG, Pasmans SG, et al. Ara h 2 is the best predictor for peanut allergy in adults. J Allergy Clin Immunol Pract. 2013;1(6):632-8 e1.
32. Kansen HM, Erp v, F.C. , Knulst AC, Ehlers AM, Lyons SA, Knol EF, et al. Accurate prediction of peanut allergy in one third of adults using a validated Ara h 2 cutoff. J Allergy Clin Immunol Pract. 2020;S2213-2198(20)31245-9.
33. Masthoff LJ, van Hoffen E, Mattsson L, Lidholm J, Andersson K, Zuidmeer-Jongejan L, et al. Peanut allergy is common among hazelnut-sensitised subjects but is not primarily the result of IgE cross-reactivity. Allergy. 2015;70(3):265-74.
34. Villalta D, Scala E, Mistrello G, Amato S, Asero R. Evidence of Cross-Reactivity between Different Seed Storage Proteins from Hazelnut (Corylus avellana) and Walnut (Juglans regia) Using Recombinant Allergen Proteins. Int Arch Allergy Immunol. 2019;178(1):89-92.
35. Costa J, Mafra I, Carrapatoso I, Oliveira MB. Hazelnut Allergens: Molecular Characterization, Detection, and Clinical Relevance. Crit Rev Food Sci Nutr. 2016;56(15):2579-605.
36. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. J Allergy Clin Immunol. 1997;100(4):444-51.
37. Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ. Flow cytometric analysis of in vitro activated basophils, specific IgE and skin tests in the diagnosis of pollen-associated food allergy. Cytometry B Clin Cytom. 2005;64(1):28-33.

38. Glaumann S, Nilsson C, Johansson SG, Asarnoj A, Wickman M, Borres MP, et al. Evaluation of basophil allergen threshold sensitivity (CD-sens) to peanut and Ara h 8 in children IgE-sensitised to Ara h 8. Clin Mol Allergy. 2015;13(1):5.
39. Wolbing F, Kunz J, Kempf WE, Grimmel C, Fischer J, Biedermann T. The clinical relevance of birch pollen profilin cross-reactivity in sensitised patients. Allergy. 2017;72(4):562-9.

Supplemental files

Table S1. Comparison of included and excluded subjects

	Included (N = 108)	**Excluded (N = 31)**	**p**
Age in years, *mean (±SD)*	32 (±13.0)	38 (±14.0)	0.046
Male sex	32 (29.6)	9 (29.0)	0.949
Asthma	56 (51.9)	14 (45.2)	0.511
AD	58 (53.7)	12 (38.7)	0.141
AR	94 (87.0)	28 (90.3)	0.623
sIgE level in kU_A/L, *median (IQR)*			
Hazelnut extract (N=101/8)*	4.60 (1.32-14.05)	5.65 (1.87-25.98)	0.482
Cor a 1 (N=100/8)*	4.55 (0.73-15.83)	8.45 (0.98-32.50)	0.561
Cor a 8 (N=90/7)*	0.00 (0.0-0.06)	0.00 (0.0-0.00)	0.193
Cor a 9 (N=100/9)*	0.00 (0.0-0.14)	0.00 (0.0-0.00)	0.058
Cor a 14 (N=101/9)*	0.00 (0.0-0.09)	0.00 (0.0-0.01)	0.124

Values are expressed as N (%) unless otherwise specified. *The number of included/excluded subjects from whom data on this measurement of IgE was available. 31 patients were excluded from analyses due to inconclusive DBPCFC (N=19) or lack of serum for determining IgE levels (N=12). *SD, standard deviation; AD, atopic dermatitis; AR, allergic rhinitis; sIgE, specific IgE; IQR, interquartile range.*

Table S2. Overview of all occurring IgE sensitisation patterns

IgE sensitisation pattern					**Total N**	**DBPCFC outcome**		
Extract	Cor a 1	Cora 8	Cor a 9	Cor a 14		No HA	HA	Obj HA
✓	✓				48	26	22	7
✓	✓		✓	✓	7	0	7	6
✓	✓			✓	6	3	3	1
✓	✓	✓		✓	5	4	1	0
✓	✓	✓			3	0	3	0
✓	✓		✓		3	1	2	0
✓		✓			2	2	0	0
✓					1	0	1	0
			✓		1	0	1	0
✓				✓	1	0	1	1
✓	✓	✓	✓		1	1	0	0
✓	✓	✓	✓	✓	1	0	1	0
78	74	12	13	20	89	43	46	18

IgE sensitisation: IgE ≥ 0.35 kU_A/L. *N, number of subjects; Obj, objective.*

Chapter 7

Walnut allergy across Europe: distribution of allergen sensitisation patterns and prediction of severity

J Allergy Clin Immunol Pract. 2020; S2213-2198(20)30935. In press.

Sarah A. Lyons, Mareen R. Datema, Thuy-My Le, Riccardo Asero, Laura Barreales, Simona Belohlavkova, Frédéric de Blay, Michael Clausen, Ruta Dubakiene, Cristina Fernández-Perez, Philipp Fritsche, David Gislason, Karin Hoffmann-Sommergruber, Monika Jedrzejczak-Czechowicz, Laurian Jongejan, Marek L. Kowalski, Tanya Kralimarkova, Jonas Lidholm, Nikolaos G. Papadopoulos, Bo Pontoppidan, Todor A. Popov, Nayade del Prado, Ashok Purohit, Isabel Reig, Suranjith L. Seneviratne, Athanassios Sinaniotis, Emilia Vassilopoulou, Serge A. Versteeg, Stefan Vieths, Aeilko H. Zwinderman, Paco M.J. Welsing, E.N. Clare Mills, Barbara Ballmer-Weber, André C. Knulst, Montserrat Fernández-Rivas*, Ronald Van Ree*

*Contributed equally

Abstract

Background
Walnut allergy is common across the globe, but data on the involvement of individual walnut components are scarce.

Objective
To identify geographical differences in walnut component sensitisation across Europe, explore co-sensitisation and cross-reactivity, and assess associations of clinical and serological determinants with severity of walnut allergy.

Methods
As part of the EuroPrevall outpatient surveys in 12 European cities, standardised clinical evaluation was conducted in 531 individuals reporting symptoms to walnut, with sensitisation to all known walnut components assessed in 202 subjects. Multivariable Lasso regression was applied to investigate predictors for walnut allergy severity.

Results
Birch pollen-related walnut sensitisation (Jug r 5) dominated in Northern and Central Europe and LTP sensitisation (Jug r 3) in Southern Europe. Profilin sensitisation (Jug r 7) was prominent throughout Europe. Sensitisation to storage proteins (Jug r 1, 2, 4 and 6) was detected in up to 10% of subjects. The walnut components that showed strong correlations with pollen and other foods differed between centres. The combination of determinants best predicting walnut allergy severity were: symptoms upon skin contact with walnut, atopic dermatitis (ever), family history of atopic disease, mugwort pollen allergy, sensitisation to cat/dog, positive SPT to walnut, and IgE to Jug r 1, 5, 7 or carbohydrate determinants (AUC = 0.81 [95%-CI 0.73-0.89]).

Conclusions
Walnut allergic subjects across Europe show clear geographical differences in walnut component sensitisation and co-sensitisation patterns. A predictive model combining results from component-based serology testing with results from extract-based testing and information on clinical background allows for good discrimination between mild-to-moderate and severe walnut allergy.

Introduction

Walnut is one of the tree nuts most often reported to elicit food allergic reactions in European countries and globally.[1-3] Ongoing developments in food allergy diagnostic testing, make it possible to assess IgE sensitisation to a broadening spectrum of specific food allergens, commonly referred to as component-resolved diagnostics (CRD). At the time of this study, seven components of the 'English' walnut, *Juglans regia*, had been characterised: Jug r 1 (2S albumin), Jug r 2 (vicilin-like 7S globulin), Jug r 3 (lipid transfer protein [LTP]), Jug r 4 (legumin-like 11S globulin), Jug r 5 (pathogenesis-related protein family 10 [PR-10] protein), Jug r 6 (vicilin-like 7S globulin), and Jug r 7 (profilin).

Studies on geographical differences in sensitisation patterns to walnut components across Europe are scarce.[4] One study investigated sensitisation to walnut components in 91 walnut-allergic patients from three European regions, and described a particularly high occurrence of Jug r 3 sensitisation in Spain, and Jug r 5 sensitisation in Germany and Switzerland.[5] However, geographical comparisons were limited by the fact that only children were included in Germany, and only adults in Switzerland. Larger studies, with standardised cross-border inclusion criteria, and a broader geographical distribution including Northern and Eastern Europe, are needed to substantiate previous findings and expand data on international comparisons.

CRD can be of help in distinguishing primary from cross-reactive walnut sensitisation,[6, 7] but also in predicting severity of food allergic reactions.[8, 9] For walnut, literature suggests that IgE to the seed storage proteins Jug r 1, Jug r 2, Jug r 4, and Jug r 6, is associated with more severe reactions, [5, 10] but data are limited. A recent study evaluated CRD data in combination with other serological measurements and clinical factors for predicting severity of hazelnut allergy, and found that a model combining IgE to Cor a 14, IgE to walnut extract, atopic dermatitis, and pollen allergy, performed well.[9] Such a predictive model has not yet been elaborated for walnut allergy.

In this study, we explored walnut allergy through data collected during the standardised EuroPrevall outpatient project, from 12 geographically, culturally and socio-economically diverse regions across Europe. Our aim was three-fold: 1. to identify differences in sensitisation patterns to walnut components across Europe; 2. to assess relationships between IgE to walnut components, and IgE to pollen and foods other than walnut, providing insight into possible primary sensitisers; and 3. to optimally predict severity of walnut allergy using data from clinical history and IgE responses to walnut and walnut components.

Methods

Study design, setting and subjects

Participants of the EuroPrevall outpatient clinic study reporting adverse reactions within 2 hours of ingestion of walnut, were evaluated in this study. A detailed methodology of the standardised EuroPrevall outpatient food allergy work-up, was published previously.[11]

Data were collected between 2006 and 2009 in 12 European allergy clinics, in Athens (Greece), Lodz (Poland), Madrid (Spain), Manchester (United Kingdom), Milan (Italy), Prague (Czech Republic), Reykjavik (Iceland), Sofia, (Bulgaria), Strasbourg (France), Utrecht (The Netherlands), Vilnius (Lithuania) and Zurich (Switzerland).

Ethical approval and written informed consent were obtained in each centre and from each participating subject.

Data collection

A detailed questionnaire was completed for each subject by a trial physician, and focused on demographic data, reaction characteristics, and personal and family history of atopy.

IgE sensitisation was assessed through skin prick test (SPT) and serum analyses, according to the same standardised approach in all centres (see details in the 'Supplemental methods on data collection'), using extracts from food (including walnut) and inhalant allergens that are commonly implicated in food allergy across Europe. Additional prick-to-prick testing (PTP) with fresh walnut was performed in case of negative SPT with walnut extract, as indicated by local practice. Additional testing of sera for IgE to walnut components Jug r 1, Jug r 2, a low-molecular-weight fragment of Jug r 2 (Jug r 2 LMW), Jug r 3, Jug r 4, Jug r 5, Jug r 6, and Jug r 7, was performed in January 2008 with all sera collected at that time. Jug r 2 LWM is described in the 'Supplemental methods on data collection'. SPT results were expressed as allergen/histamine wheal ratios, and a ratio ≥0.5 was considered positive. IgE levels ≥0.35 kU_A/L were considered positive.

Definitions

Probable walnut allergy was defined as a combination of reported symptoms to walnut and matching IgE sensitisation, as demonstrated by a positive walnut SPT, PTP, and/or presence of serum IgE against walnut extract and/or 1 or more individual walnut components as tested by ImmunoCAP.

Reactions to walnut were classified as *severe* if subjects reported dysphagia, dysphonia, lower airway, cardiovascular, or neurological symptoms, or anaphylaxis (specifically severe laryngeal oedema, severe bronchospasm, or hypotensive shock). All other symptoms were considered *mild-to-moderate*: isolated oral allergy symptoms, symptoms of the skin, eyes, upper airway, or gastro-intestinal system (see details in the 'Supplemental methods on data collection').[12, 13]

Allergy to inhalant allergen sources and to latex was defined as symptoms and matching IgE sensitisation in SPT and/or ImmunoCAP to the respective allergen source.

Statistical analyses

Walnut sensitisation patterns across Europe

Demographics, reaction severity, and proportions of positive test results, were explored for each participating centre. Medians and interquartile ranges were calculated to evaluate IgE levels for walnut extract and walnut components. Differences between centres in levels of IgE to walnut extract were tested using the Kruskal-Wallis test with Bonferroni correction.

Relationship between IgE to walnut components and other allergens

Spearman rho coefficients were calculated to evaluate relationships between levels of IgE to walnut components, and levels of IgE to food, latex, and pollen extracts. Bonferroni correction was used to correct for multiple comparisons.

Predictors for severity of walnut allergy

Only subjects conforming to the definition of 'probable walnut allergy' were included for prediction of severity of walnut allergy. Univariable logistic regression was performed to explore crude associations between demographics, clinical background variables, walnut sensitisation patterns, and severity of walnut allergy.

To identify the most discriminative combination of predictors for severity of walnut allergy, Least Absolute Shrinkage and Selection Operator (Lasso) regression was applied. Lasso regression is a form of penalised regression, which selects only the most contributive predictors, and applies shrinkage of regression coefficients through cross-validation, to limit overfitting.[14] In order to enable the use of all data and increase power for this predictive analysis, multiple imputation of sporadically missing data on predictor variables was performed (10 imputations by Chained Equations using the R package *mice*).[15] Missing data is described in Table S1.

A three-step approach to model building was taken. In model I, demographic and clinical variables were entered, and Lasso regression selected the most discriminative combination of predictors. In model II, variables on IgE sensitisation to walnut extract

as assessed by SPT and ImmunoCAP were entered, along with the variables selected in model I. In model III, ImmunoCAP results for walnut components, and IgE to Ana c 2 (bromelain) as a measure for cross-reactive carbohydrate determinants (CCD), were added to the variables remaining after selection in model II. Predictor variables selected in at least 7 of the 10 imputed datasets were included in each model, and their coefficients and 95% confidence intervals (CI) were pooled, using Rubin's rules. To assess how well each model could discriminate between mild-to-moderate and severe walnut allergy, the area under the curves (AUC) of the receiving operating characteristics (ROC) and corresponding 95%-CIs were calculated and pooled over the 10 imputed datasets. DeLong's test was used to compare AUC values.[16] Analyses were conducted with SPSS version 25 and R version 3.4.1.

Results

Population characteristics

As the fourth most commonly reported causative food in the EuroPrevall outpatient clinic study, walnut was reported to elicit symptoms in 531 (23.4%) subjects, most often in Utrecht (37.0%) and least often in Reykjavik (6.3%). Most were female (64.8%) and over 18 years of age (84.6%) (Table 1). The most commonly reported symptoms were oral allergy symptoms in 426/531 (80.2%) subjects, of which 214 had no other symptoms. Symptoms of the upper airway, skin and digestive system were reported by respectively 33.3%, 32.0% and 23.2% of subjects. Fewer subjects reported lower airway (15.1%), cardiovascular (2.4%), or neurological (3.2%) symptoms. Anaphylaxis was reported by 15 subjects (2.8%).

Walnut sensitisation patterns across Europe

SPT and ImmunoCAP with walnut extract were positive in 40.8% and 35.5% of subjects (Table 1). Positive serology to walnut extract was found in less than 30% of subjects reporting symptoms to walnut from Lodz, Strasbourg, Utrecht, and Zurich, but in more than 80% of subjects from Athens and Madrid. In subjects with positive serology to walnut extract, median IgE levels were lowest in Strasbourg, Sofia and Manchester, and highest in Milan, Lodz, Utrecht, Prague and Athens (Figure 1).

Sensitisation by CRD was assessed in 202 subjects, and 79.4% of the 199 subjects with complete CRD results were found to be sensitised to at least 1 individual walnut component by ImmunoCAP. The distribution of IgE levels in subjects sensitised to a specific walnut component is shown in Figure 2. Median IgE levels for PR-10 protein Jug r 5 were highest. Of the subjects with *negative* SPT and ImmunoCAP to walnut extract (N=237), in whom CRD with all walnut components was completed (N=79), 70.9% were sensitised to at least 1 component (N=56 of 79), most frequently to Jug r 5 (N=50 of 79, 63.3%) (Table S2).

Table 1. Characteristics of subjects with self-reported walnut allergy across Europe

	Total (N=531)	**Athens (N=44)**	**Madrid (N=25)**	**Manchester (N=30)**	**Milan (N=39)**	**Lodz (N=74)**	**Prague (N=19)**
Demographics							
Age in years, *mean (±SD)*	30.4 (±13.9)	27.8 (±10.3)	23.8 (±12.9)	30.7 (±13.3)	34.7 (±10.9)	29.5 (±18.4)	15.9 (±11.7)
Age <18 years	82 (15.4)	4 (9.1)	7 (28.0)	5 (16.7)	0 (0.0)	22 (29.7)	11 (57.9)
Female sex	344 (64.8)	17 (38.6)	18 (72.0)	23 (76.7)	29 (74.4)	59 (79.7)	10 (52.6)
Symptom severity*							
Mild	214 (40.3)	14 (31.8)	9 (36.0)	3 (10.0)	27 (69.2)	14 (18.9)	5 (26.3)
Moderate	184 (34.7)	18 (40.9)	9 (36.0)	15 (50.0)	6 (15.4)	41 (55.4)	6 (31.6)
Severe	133 (25.0)	12 (27.3)	7 (28.0)	12 (40.0)	6 (15.4)	19 (25.7)	8 (42.1)
Sensitisation to walnut**							
SPT walnut extract positive	211 (40.8)	36 (81.8)	13 (54.2)	9 (30.0)	21 (53.8)	12 (16.9)	7 (38.9)
ImmunoCAP walnut extract positive	182 (35.5)	35 (81.4)	20 (87.0)	11 (39.3)	19 (48.7)	10 (13.9)	7 (43.8)
CRD walnut performed, *N*	202	19	13	5	18	15	8
CRD walnut positive***	158 (79.4)	13 (68.4)	10 (76.9)	4 (80.0)	15 (83.3)	9 (64.3)	8 (100.0)
Jug r 1	21 (10.4)	0 (0.0)	3 (23.1)	1 (20.0)	0 (0.0)	1 (6.7)	3 (37.5)
Jug r 2	19 (9.6)	0 (0.0)	3 (23.1)	1 (20.0)	1 (5.6)	1 (7.1)	2 (25.0)
Jug r 2 LMW	43 (22.1)	5 (26.3)	4 (30.8)	1(20.0)	3 (16.7)	3 (23.1)	3 (37.5)
Jug r 3	28 (13.9)	9 (47.4)	3 (23.1)	0 (0.0)	4 (22.2)	0 (0.0)	2 (25.0)
Jug r 4	18 (9.2)	0 (0.0)	3 (23.1)	2 (40.0)	1 (5.6)	0 (0.0)	2 (25.0)
Jug r 5	115 (58.1)	1 (5.3)	1 (7.7)	1 (20.0)	12 (66.7)	7 (50.0)	7 (87.5)
Jug r 6	12 (6.2)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (25.0)
Jug r 7	47 (23.3)	4 (21.1)	4 (30.8)	0 (0.0)	7 (38.9)	1 (6.7)	1 (12.5)
Probable walnut allergy	336 (65.8)	43 (97.7)	23 (95.8)	15 (53.6)	32 (82.1)	22 (31.4)	13 (81.3)

Table 1. Characteristics of subjects with self-reported walnut allergy across Europe (continued)

	Reykjavik (N=9)	Sofia (N=10)	Strasbourg (N=50)	Utrecht (N=74)	Vilnius (N=50)	Zurich (N=107)	p
Demographics							
Age in years, *mean (±SD)*	36.4 (±17.6)	23.2 (±14.3)	33.8 (±12.8)	31.2 (±11.3)	27.9 (±14.0)	33.8 (±12.8)	<0.001
Age <18 years	1 (11.1)	4 (40.0)	5 (10.0)	3 (4.1)	14 (28.0)	6 (5.6)	<0.001
Female sex	6 (66.7)	7 (70.0)	34 (68.0)	54 (73.0)	22 (44.0)	65 (60.7)	<0.001
Symptom severity*							
Mild	2 (22.2)	0 (0.0)	33 (66.0)	33 (44.6)	18 (36.0)	56 (52.3)	<0.001
Moderate	2 (22.2)	7 (70.0)	9 (18.0)	20 (27.0)	22 (44.0)	29 (27.1)	
Severe	5 (55.6)	3 (30.0)	8 (16.0)	21 (28.4)	10 (20.0)	22 (20.6)	
Walnut sensitisation**							
SPT walnut extract positive	4 (44.4)	2 (20.0)	13 (26.5)	25 (37.3)	38 (77.6)	31 (29.0)	<0.001
ImmunoCAP walnut extract positive	3 (33.3)	3 (30.0)	13 (26.5)	19 (25.7)	15 (34.1)	27 (25.5)	<0.001
CRD walnut performed, *N*	3	4	16	20	14	67	
CRD walnut positive***	1 (33.3)	1 (33.3)	15 (93.8)	19 (95.0)	11 (84.6)	52 (77.6)	0.065
Jug r 1	1 (33.3)	0 (0.0)	1 (6.3)	7 (35.0)	0 (0.0)	4 (6.0)	0.001
Jug r 2	1 (33.3)	1 (25.0)	1 (6.7)	6 (30.0)	1 (7.7)	1 (1.5)	0.007
Jug r 2 LMW	1 (33.3)	1 (33.3)	3 (20.0)	8 (40.0)	3 (23.1)	8 (12.3)	0.527
Jug r 3	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.0)	1 (7.1)	6 (9.0)	0.002
Jug r 4	1 (33.3)	0 (0.0)	1 (6.7)	6 (30.0)	0 (0.0)	2 (3.1)	0.001
Jug r 5	0 (0.0)	0 (0.0)	14 (93.3)	18 (90.0)	11 (84.6)	43 (62.5)	<0.001
Jug r 6	1 (33.3)	0 (0.0)	0 (0.0)	5 (25.0)	0 (0.0)	3 (4.6)	0.005
Jug r 7	0 (0.0)	0 (0.0)	1 (6.3)	9 (45.0)	1 (7.1)	19 (28.4)	0.041
Probable walnut allergy	5 (55.6)	5 (50.0)	29 (60.4)	37 (54.4)	42 (85.7)	70 (66.0)	<0.001

All measurements are in N (%) unless otherwise specified. *Mild: isolated oral allergy symptoms. Moderate: symptoms of the skin, eyes, upper airway, or gastro-intestinal system. Severe: dysphagia, dysphonia, lower respiratory, cardiovascular, or neurological symptoms, or anaphylaxis. **The results show the number and percentage of subjects with positive sensitisation according to each test. SPT was considered positive if allergen/histamine wheal ratio ≥0.5; ImmunoCAP if IgE ≥0.35 kU_A/L. SPT with walnut extract was performed in 517 subjects; ImmunoCAP with walnut extract in 513 subjects.***For some centres (Lodz, Sofia, Strasbourg, Vilnius, and Zurich), the results of 1 or 2 of the individual CRD tests were missing. The percentage given in brackets is the percentage of the total number of available CRD results. The p-values pertain to comparison of centres and were determined for exploratory purposes (no correction for multiple testing) using the Pearson chi-square test for categorical variables, and the Anova or Kruskall-Wallis test for continuous variables. *CRD, component-resolved diagnostics; SPT, skin prick test.*

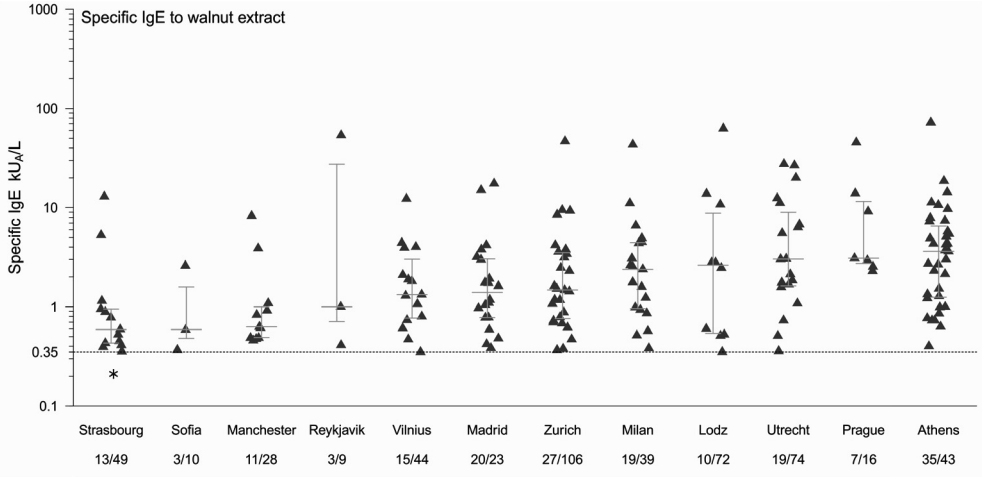

Figure 1. IgE to walnut extract across Europe
Walnut specific IgE levels in subjects with positive serology to walnut extract in ImmunoCAP (≥0.35 kU_A/L). The triangles represent individual subjects, the lines indicate medians and interquartile ranges. n/N = number of subjects with positive serology/ number of subjects in whom ImmunoCAP with walnut extract was performed. *Significantly different from Prague, Athens and Utrecht.

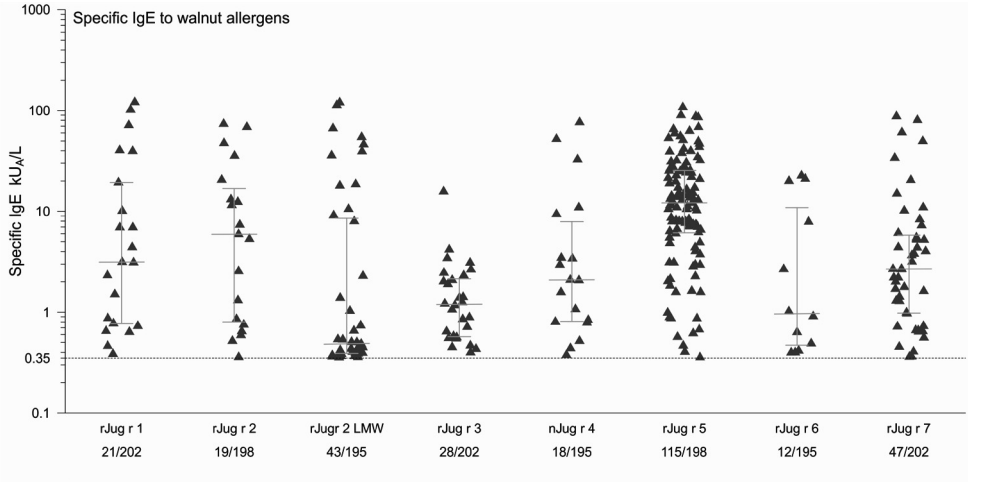

Figure 2. IgE to walnut allergens
Walnut allergen specific IgE levels in subjects with positive serology to the respective walnut allergens in ImmunoCAP (≥0.35kU_A/L). The triangles represent individual subjects, the lines indicate medians and interquartile ranges. n/N = number of subjects with positive serology/ number of subjects in whom ImmunoCAP with walnut allergen was performed.

For international comparison of walnut component sensitisation patterns, only centres where CRD results were available for at least 10 subjects were taken into account (Table 1, Figure 3). Sensitisation to PR-10 protein Jug r 5 was most prevalent everywhere except in Athens and Madrid. In Athens, sensitisation to LTP Jug r 3 dominated. Besides Athens, LTP sensitisation occurred most frequently in other Southern centres, Madrid and Milan. Sensitisation to profilin Jug r 7 was most common after sensitisation to Jug r 5, and was particularly recognised in Utrecht, Milan, Madrid, Zurich and Athens. Storage proteins Jug r 1, 2, 4 and 6 were recognised in up to 10% of subjects overall; all most frequently in Utrecht, followed by Madrid.

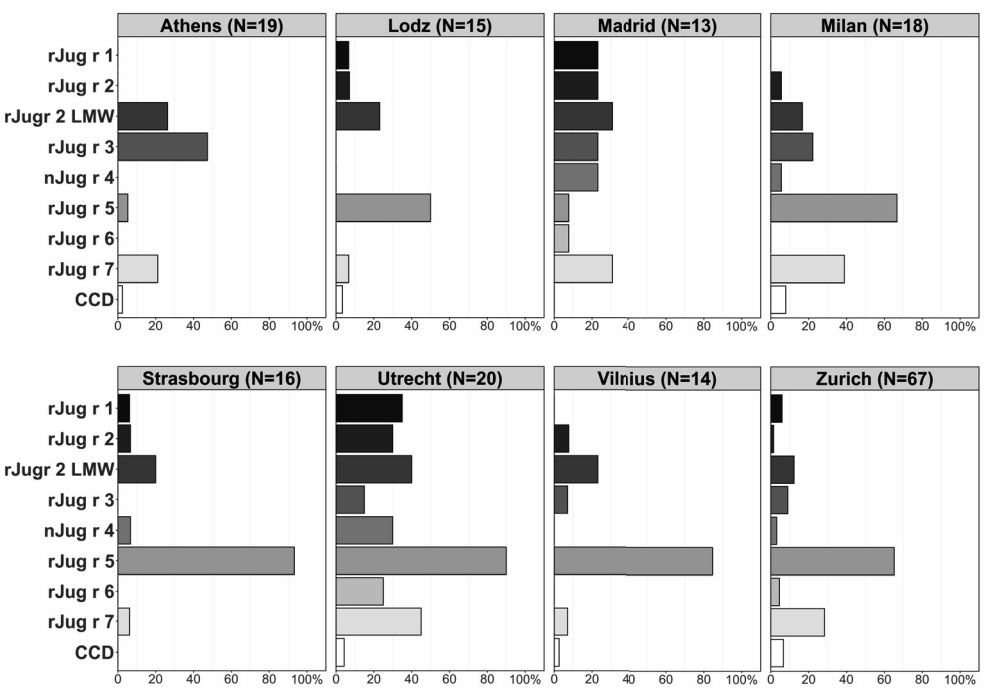

Figure 3. IgE sensitisation to walnut components across Europe
N = the total number of subjects in whom CRD was performed. The number of subjects in whom CRD was positive (≥0.35 kU_A/L), is visible for each centre in Table 1. Only centres where CRD was completed in at least 10 subjects, are shown. The length of the bars corresponds with the percentage of subjects with positive serology to each specific walnut allergen.

Relationship between IgE to walnut components and other allergens

Figure 4 and E1 reveal how IgE levels to walnut components correlated with IgE levels to pollen and other foods.

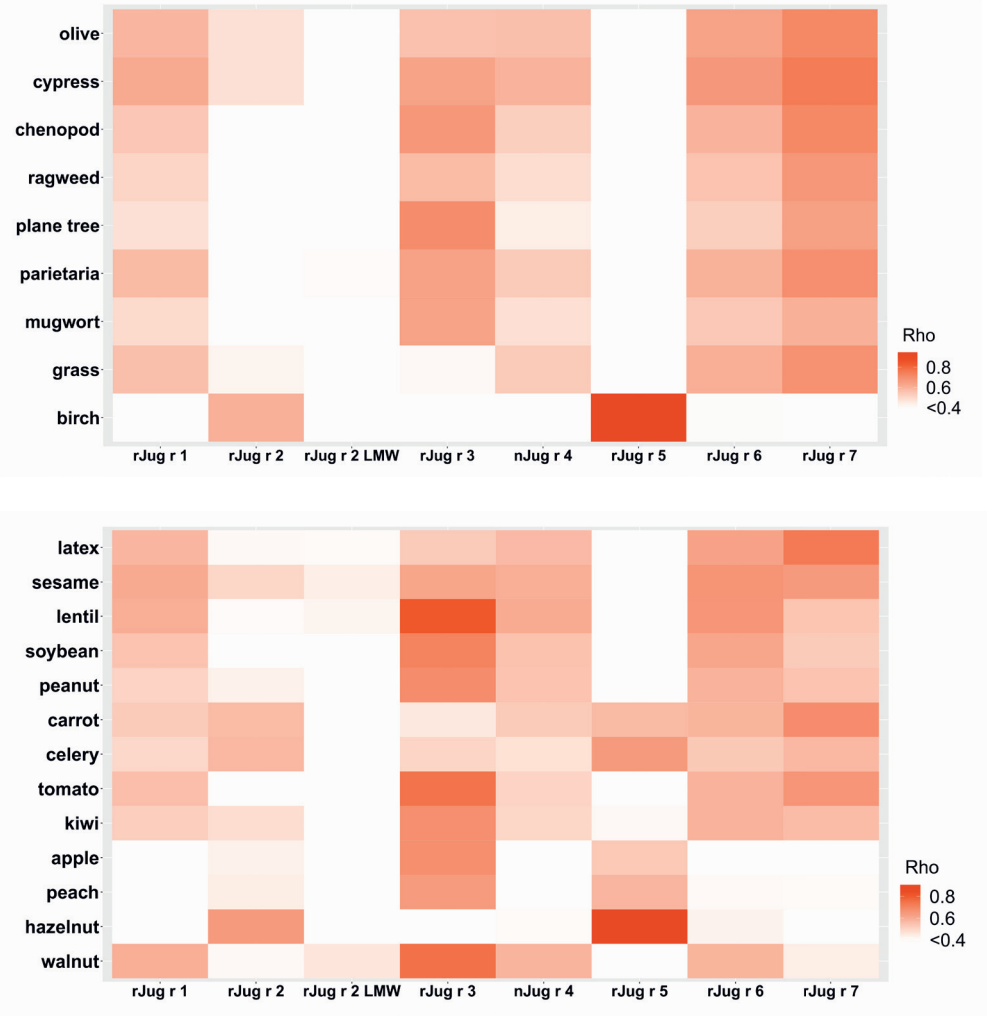

Figure 4. Correlation between IgE levels to walnut components and pollen and other foods
The numeric values of the Spearman rho correlation coefficients are available from Table S3.

Regarding pollen, the strongest correlation overall was between IgE to Jug r 5 and birch (Table S3, ρ=0.92). This positive correlation was prominent in all evaluated centres (ρ=0.75-0.97), except Madrid and Athens. In Madrid, the strongest correlation between a walnut component and pollen, was between Jug r 7 and grass pollen (ρ=0.70). In Athens, the correlations between Jug r 3 and mugwort, *Chenopodium*, and plane tree pollen (ρ=0.76-0.86), were most remarkable.

Regarding IgE levels to food extracts other than walnut, the overall strongest correlations were found between Jug r 5 and hazelnut (ρ=0.88), and between Jug r 3 and lentil (ρ=0.80). However, the walnut components most likely to show strong correlations with the various foods differed per centre (Table S4). For example, IgE levels to hazelnut correlated strongly with Jug r 5 IgE levels in most centres, but with Jug r 3 IgE levels in Athens. Lentil IgE levels were found to correlate strongly with different walnut components in each centre, but never with Jug r 5 or Jug r 7.

Predictors for severity of walnut allergy

Probable walnut allergy, where reported symptoms were supported by IgE sensitisation, was identified in 336 subjects (Table 1). Of these 336 subjects, 246 (73.2%) had mild-to-moderate symptoms, and 90 (26.8%) had severe symptoms. The results from univariable analyses are listed in Table 2. Regarding clinical background, subjects with severe walnut allergy were significantly more likely to have mugwort allergy, and significantly less likely to have birch pollen allergy or IgE sensitisation to cat or dog, than subjects with mild-to-moderate walnut allergy. Although not statistically significant, severely allergic subjects were more often sensitised to walnut in SPT, and had higher median IgE levels to walnut extract in ImmunoCAP. No significant differences between severity groups were found regarding the percentage of subjects sensitised to specific walnut allergens, or median IgE levels, although trends amongst sensitised subjects suggested higher IgE levels to storage proteins and LTP in severely allergic and to PR-10 and profilin in mild-to-moderately allergic subjects (Table S5).

CRD was performed in 177 of 336 subjects with probable walnut allergy. These 177 subjects were included in the multivariable analyses for prediction of severity of walnut allergy. Table 3 presents the results of the Lasso regression analysis. Of all the demographics and clinical variables included in model I, Lasso regression selected 'symptoms upon skin contact with walnut', 'family history of atopic disease', 'atopic dermatitis', and 'mugwort pollen allergy', which were positively associated with severe walnut allergy, and 'IgE sensitisation to cat or dog', which was inversely associated with severe walnut allergy. In model II, all the variables selected in model I remained. Additionally, SPT positivity to walnut was selected as an extra predictor (positive association). Finally, in model III, IgE levels to Jug r 1, Jug r 5, Jug r 7, and Ana c 2 were found to further contribute to prediction of severity of walnut allergy. Although walnut SPT positivity was selected as an additional predictor in model II, model accuracy remained similar to model I (AUC 0.74 in both models). Addition of CRD in model III significantly increased the AUC to 0.81 (p_{DeLong}=0.002). Additional analyses of the performance of individual tests revealed that combinations of tests as defined in the Lasso models, predicted severity better than SPT or ImmunoCAP to walnut extract, or ImmunoCAP to individual walnut allergens (evaluated separately or combined), for which AUCs ranged from 0.48 to 0.66 (Table S6).

Table 2. Characteristics of subjects with probable walnut allergy in relation to symptom severity

	Mild-to-moderate (N=246)	Severe (N=90)	p	Univariable OR [95%-CI]
Demographics				
Age in years, *mean (±SD)*	29.9 (±13.0)	28.4 (±12.5)	0.972	0.99 [0.97-1.01]
Female sex	147 (59.8)	47 (52.2)	0.216	0.74 [0.45-1.98]
Clinical background				
Age onset of symptoms < 14 years	97 (39.8)	38 (42.2)	0.683	1.11 [0.67-1.81]
Symptoms upon skin contact with walnut	9 (4.1)	7 (8.8)	0.117	2.23 [0.77-6.19]
Family history of atopic disease	152 (67.6)	60 (71.4)	0.514	1.20 [0.70-2.11]
Atopic dermatitis (ever)	68 (28.2)	32 (36.4)	0.155	1.45 [0.86-2.43]
Asthma (ever)	229 (97.0)	86 (96.6)	0.851	0.88 [0.24-4.14]
Birch pollen allergy	153 (64.6)	44 (51.8)	0.038	0.59 [0.36-0.97]
Grass pollen allergy	138 (58.5)	53 (62.4)	0.532	1.18 [0.71-1.97]
Mugwort pollen allergy	31 (13.3)	20 (23.0)	0.035	1.95 [1.03-3.62]
Planetree pollen allergy	17 (7.4)	8 (9.2)	0.595	1.27 [0.50-2.97]
House dust mite allergy	66 (28.1)	23 (26.7)	0.812	0.94 [0.53-1.61]
Latex allergy	12 (5.1)	5 (5.7)	0.813	1.14 [0.35-3.17]
Cat/dog sensitisation	173 (73.6)	53 (60.9)	0.027	0.56 [0.33-0.94]
Walnut sensitisation				
SPT walnut extract positive*	150 (61.5)	61 (68.5)	0.236	1.37 [0.82-2.31]
IgE level walnut extract	0.39 (0.05-1.70)	0.73 (0.15-3.63)	0.018	1.02 [0.99-1.05]
IgE level Jug r 1	0.01 (0.00-0.06)	0.01 (0.00-0.05)	0.719	1.00 [0.95-1.02]
IgE level Jug r 2	0.05 (0.02-0.13)	0.04 (0.01-0.08)	0.516	1.02 [0.98-1.06]
IgE level Jug r 2 LMW	0.24 (0.17-0.36)	0.23 (0.15-0.32)	0.571	1.01 [0.99-1.04]
IgE level Jug r 3	0.04 (0.01-0.17)	0.05 (0.01-0.12)	0.739	0.93 [0.54-1.21]
IgE level Jug r 4	0.03 (0.01-0.09)	0.02 (0.01-0.06)	0.215	1.00 [0.93-1.05]
IgE level Jug r 5	6.69 (0.03-16.83)	1.60 (0.02-9.11)	0.118	0.97 [0.94-1.00]
IgE level Jug r 6	0.03 (0.01-0.07)	0.02 (0.01-0.07)	0.399	1.04 [0.91-1.16]
IgE level Jug r 7	0.02 (0.00-0.65)	0.02 (0.00-0.18)	0.503	0.92 [0.75-1.00]

All measurements are in N (%) or median (Q1-Q3) unless otherwise specified. All IgE levels were measured in kU_A/L on ImmunoCAP. For subjects with mild-to-moderate and severe probable walnut allergy, SPT was performed in respectively 244 and 89 subjects; ImmunoCAP with walnut extract in 240 and 89 subjects; and CRD in 136 and 41 subjects. *SPT was considered positive if allergen/histamine wheal ratio ≥0.5. *CI, confidence interval; OR, odds ratio; SPT, skin prick test.*

Table 3. Prediction models for severity of walnut allergy

	Model I: Demographics & clinical background		**Model II:** Model I + sensitisation to walnut extract		**Model III:** Model II + sensitisation to walnut components	
	OR	**95%-CI**	**OR**	**95%-CI**	**OR**	**95%-CI**
Symptoms upon skin contact	1.95	1.51-2.53	2.32	1.48-3.63	2.43	1.58-3.75
Family history atopic disease	1.65	1.49-1.82	1.97	1.74-2.23	2.69	2.35-3.07
Atopic dermatitis	1.89	1.64-2.19	2.12	1.82-2.48	2.68	2.26-3.18
Mugwort pollen allergy	1.96	1.66-2.32	2.28	1.93-2.69	3.75	3.18-4.42
Cat/dog sensitisation	0.41	0.36-0.48	0.34	0.30-0.40	0.40	0.35-0.46
SPT walnut positive			1.06	0.94-1.18	1.07	0.96-1.20
IgE level Jug r 1					0.99	0.98-1.00
IgE level Jug r 5					0.97	0.97-0.97
IgE level Jug r 7					0.98	0.97-0.98
IgE level Ana c 2					0.63	0.55-0.73
Intercept	*-1.32*		*-1.45*		*-1.52*	
AUC (95%-CI)	0.74 (0.65-0.83)		0.74 (0.65-0.83)		0.81 (0.73-0.89)	

All IgE levels were measured in kU_A/L on ImmunoCAP. The 95% confidence intervals (CI) for each coefficient were calculated from standard errors obtained for each imputed datasets through bootstrapping, and pooled over the 10 imputed datasets using Rubin's rules. Unselected variables model I: age, sex, age at onset of symptoms to walnut (<14 *versus* ≥ 14 years), asthma, birch/ grass/ plane tree pollen allergy, house dust mite allergy, latex allergy. Unselected variables model II: IgE level walnut extract. Unselected variables model III: IgE level Jug r 2, Jug r 3, Jugr4, and Jug r 6. *CI, confidence interval; OR, odds ratio; SPT, skin prick test.*

Discussion

The current study is the largest European multicentre study on walnut allergy to date. Clear geographical differences were observed in walnut component sensitisation and co-sensitisation patterns, and our predictive model combining demographic, clinical, and serological variables attained good accuracy with an AUC of 0.81 for distinguishing mild-to-moderate from severe walnut allergy.

Walnut allergy across Europe: Allergen (co-)sensitisation patterns

The distribution of sensitisation to walnut components across Europe was found to follow the same pattern as many other plant source foods, including other tree nuts[17]: sensitisation to PR-10 proteins (Jug r 5) in Northern and Central Europe;[18] sensitisation to profilin (Jug r 7) throughout Europe,[19] and sensitisation to lipid transfer proteins (Jug r 3) in the Mediterranean.[20]

The highest overall sensitisation rates were found for Jug r 5 and Jug r 7. Pollen exposure helps explain their geographical distribution, as sensitisation to plant food PR-10 proteins and profilins is induced by similar proteins in pollen.[6, 21] Jug r 5 is homologous with Bet v 1, the major allergen of birch pollen, the dominating pollen

in Northern and Central Europe.[18] Jug r 7 sensitisation, on the other hand, could be secondary to sensitisation to almost any type of pollen, as all pollen contains profilin. Our findings were consistent with these patterns of cross-reactivity (Figure 4, Table S3): IgE to Jug r 5 showed strong correlations with IgE to birch pollen (ρ=0.92), and IgE to Jug r 7 moderate-to-strong correlations (ρ>0.60) with IgE to almost all pollen.

Sensitisation to Jug r 3 is generally thought to occur through peach as primary sensitiser,[20, 22-24] although plane tree and mugwort pollen have also been suggested as primary sources of sensitisation to LTP.[25-27] Indeed, IgE to Jug r 3 correlated with IgE to peach, plane tree, and mugwort in our data (ρ>0.60), but also to other LTP-containing pollen (e.g. *Chenopodium, Parietaria,* cypress), fruits (tomato, apple, kiwi), and legumes (lentil, soybean, peanut).[20] Future studies with IgE inhibition assays could help further differentiate between independent co-sensitisation and cross-reactivity, and identify primary sources of sensitisation to Jug r 3 and other walnut components.

Similar distributions of Jug r 3 and Jug r 5 sensitisation were observed by Ballmer-Weber *et al*. in Germany, Switzerland and Spain.[5] However, occurrence of sensitisation to walnut storage proteins was more frequent in their data (48-57%) than in ours (7-10%). This is likely due to the diverse study populations, which in the study of Ballmer-Weber *et al*. included more severely allergic subjects, more paediatric subjects, and more subjects with onset of symptoms before the age of 14 years, all of which make primary sensitisation more likely.

Notably, a high proportion of subjects sensitised to Jug r 5 tested negative to walnut extract (Table 1 and E2), as has also been observed previously.[28] This finding substantiates that the concentration of Jug r 5 is low in walnut extract, causing a low sensitivity of extract-based tests for subjects with birch pollen-related walnut allergy.

Walnut allergy across Europe: Prediction of severity

A model combining symptoms upon skin contact with walnut, history of atopic dermatitis, family history of atopic disease, mugwort pollen allergy, sensitisation to cat or dog, positive SPT for walnut, and IgE to Jug r 1, Jug r 5, Jug r 7 and CCD, was found to have the highest accuracy for predicting severity of walnut allergy (AUC 0.81 [95%-CI 0.73-0.89]).

Our findings suggest that sensitisation via the cutaneous route may be associated with severity of walnut allergy. Several studies have established that atopic dermatitis predisposes to food sensitisation and allergy, presumably as a result of skin barrier impairement.[29] In line with our findings, having atopic dermatitis was previously found to be associated with severe hazelnut allergy.[9] One could speculate that

sensitisation via the skin leads to primary (non-cross-reactive) food sensitisation, which is thought to be associated with more severe reactions.[30]

In cross-reactive food allergy, pollen is generally the primary sensitiser, with sensitisation most probably occurring through the respiratory tract. Symptomatic subjects generally present with mild symptoms.[18, 21] As remarked previously, subjects with a birch pollen-related walnut allergy are poorly detected by diagnostic tests with walnut extract, explaining the positive association between SPT and severe walnut allergy.

Remarkably, mugwort pollen allergy almost quadrupled the odds of severe walnut allergy. LTP sensitisation, which is associated with severe allergic reactions to plant source foods,[31] could be the link. It has been suggested that sensitisation to mugwort LTP (Art v 3) can facilitate subsequent sensitisation to LTP in plant source foods, and the other way around.[26, 32] However, the observation that Jug r 3 IgE levels were not predictive of walnut allergy severity, makes this explanation less likely. Another plausible explanation is that other still uncharacterised mugwort allergens are associated with severe walnut allergy.

Addition of walnut component testing was found to considerably improve prediction of walnut allergy severity. Our expectations were that sensitisation to PR-10 proteins and profilins would be associated with mild-to-moderate walnut allergy, and sensitisation to seed storage proteins and LTPs would predict severe walnut allergy.[5, 6, 9] The former associations were indeed confirmed in our data; IgE levels to Jug r 5 and 7 were predictive of mild-to-moderate walnut allergy. IgE to walnut storage proteins appears to be of lesser importance in prediction of walnut allergy severity in subjects from the general population, in whom such sensitisation occurs infrequently. We have no clear explanation for why IgE to Jug r 1 was inversely associated with severity in our data.

Overall, the prediction models in this study provide insight into the clinical profiles of subjects more likely to have mild-to-moderate or severe reactions to walnut, and suggest some particular focus areas during diagnostic work-up of walnut allergy. Besides obtaining information on allergic comorbidities and family atopy, as is standard in clinical history for food allergy, physicians assessing walnut allergy should find out if presenting patients are allergic to mugwort or have symptoms elicited by skin contact with walnut. Information on cross-reactive sensitisation (Jug r 5, Jug r 7, CCD) contributes to prediction of a more mild phenotype. As Jug r 5 is underrepresented in walnut extract, diagnostic work-up in birch-endemic areas would benefit from additional testing of Jug r 5. After validation, the prediction of a mild-to-moderate phenotype using our final model could potentially translate into performance of fewer challenge tests in clinical practice (Table S6).

Strengths and limitations

All in all, this is the largest study to map walnut sensitisation across Europe. The consistent and standardised approach to data collection makes our results particularly valuable. We did not include subjects with walnut allergy determined by food challenge, but all subjects presenting to an allergy clinic with symptoms to walnut within 2 hours of ingestion, and corresponding IgE sensitisation. Through this approach, we likely captured more subjects with pollen-related walnut allergy, who form a significant proportion of walnut allergic subjects in Europe. We have also, for the first time, suggested a prediction model for assessing severity of walnut allergy, taking both clinical evaluation and serology testing into account. The main limitation of our study was that complete CRD data were available for only 177 of 336 walnut allergic subjects. Multiple imputation and penalised regression were applied to appropriately deal with sparse data, and model I and II were also developed in the total population of 336 walnut-allergic subjects, revealing no relevant differences. However, it is important to realise that we could not adjust the multivariable analyses for centre due to sparsity of data. Although we do not expect the effect of predictors on severity to depend on centre, we do observe geographically varying baseline prevalence of severe walnut allergy (Table 1).

Conclusions

To conclude, we confirm that cross-reactivity with pollen is a major cause of walnut sensitisation and allergy across Europe, leading to molecular recognition patterns similar to those of other plant source foods. PR-10 protein and profilin sensitisation occur frequently, and predict a mild-to-moderate walnut allergy phenotype. Sensitisation to walnut storage proteins is less common. The information obtained from walnut CRD, in combination with results from extract-based testing and clinical background evaluation, allows for good discrimination between mild-to-moderate and severe walnut allergy. A prediction model combining this information performs significantly better than CRD, extract-based testing or clinical background alone.

Acknowledgements

We thank all the patients for their participation in the study. We would like to thank ALK Abello (Madrid, Spain) for their generous gift of SPT reagents. We acknowledge the support by the 6th and 7th Framework Programmes of the EU, for EuroPrevall (FP6-FOOD-CT-2005-514000) and iFAAM (Grant agreement no. 312147), respectively.

References

1. McWilliam V, Koplin J, Lodge C, Tang M, Dharmage S, Allen K. The Prevalence of Tree Nut Allergy: A Systematic Review. Curr Allergy Asthma Rep. 2015;15(9):54.
2. Burney PG, Potts J, Kummeling I, Mills EN, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitisation in European adults. Allergy. 2014;69(3):365-71.
3. Lyons SA, Burney PGJ, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, Clausen M, et al. Food Allergy in Adults: Substantial Variation in Prevalence and Causative Foods Across Europe. J Allergy Clin Immunol Pract. 2019;7(6):1920-8 e11
4. McWilliam VL, Perrett KP, Dang T, Peters RL. Prevalence and natural history of tree nut allergy. Ann Allergy Asthma Immunol. 2020;124(5):466-472
5. Ballmer-Weber BK, Lidholm J, Lange L, Pascal M, Lang C, Gernert S, et al. Allergen Recognition Patterns in Walnut Allergy Are Age Dependent and Correlate with the Severity of Allergic Reactions. J Allergy Clin Immunol Pract. 2019;7(5):1560-7 e6.
6. Blankestijn MA, Knulst AC, Knol EF, Le TM, Rockmann H, Otten HG, et al. Sensitisation to PR-10 proteins is indicative of distinctive sensitisation patterns in adults with a suspected food allergy. Clin Transl Allergy. 2017;7:42.
7. Popescu FD. Cross-reactivity between aeroallergens and food allergens. World J Methodol. 2015;5(2):31-50.
8. Masthoff LJ, Mattsson L, Zuidmeer-Jongejan L, Lidholm J, Andersson K, Akkerdaas JH, et al. Sensitisation to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. J Allergy Clin Immunol. 2013;132(2):393-9.
9. Datema MR, van Ree R, Asero R, Barreales L, Belohlavkova S, de Blay F, et al. Component-resolved diagnosis and beyond: Multivariable regression models to predict severity of hazelnut allergy. Allergy. 2018;73(3):549-59.
10. Blankestijn MA, Blom WM, Otten HG, Baumert JL, Taylor SL, Bruijnzeel-Koomen CA, et al. Specific IgE to Jug r 1 has no additional value compared with extract-based testing in diagnosing walnut allergy in adults. J Allergy Clin Immunol. 2017;139(2):688-90 e4.
11. Fernandez-Rivas M, Barreales L, Mackie AR, Fritsche P, Vazquez-Cortes S, Jedrzejczak-Czechowicz M, et al. The EuroPrevall outpatient clinic study on food allergy: background and methodology. Allergy. 2015;70(5):576-84.
12. Muraro A, Fernandez-Rivas M, Beyer K, Cardona V, Clark A, Eller E, et al. The urgent need for a harmonized severity scoring system for acute allergic reactions. Allergy. 2018;73(9):1792-800.
13. Sampson HA. Anaphylaxis and emergency treatment. Pediatrics. 2003;111(6 Pt 3):1601-8.
14. Tibshirani R. Regression Shrinkage and Selection via the Lasso. J Roy Stat Soc B. 1996;58(1):267-88.
15. Buuren vS, Groothuis-Oudshoorn K. MICE: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software. 2011;45(3):1-67.
16. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988;44(3):837-45.
17. Datema MR, Zuidmeer-Jongejan L, Asero R, Barreales L, Belohlavkova S, de Blay F, et al. Hazelnut allergy across Europe dissected molecularly: A EuroPrevall outpatient clinic survey. J Allergy Clin Immunol. 2015;136(2):382-91.
18. Werfel T, Asero R, Ballmer-Weber BK, Beyer K, Enrique E, Knulst AC, et al. Position paper of the EAACI: food allergy due to immunological cross-reactions with common inhalant allergens. Allergy. 2015;70(9):1079-90.
19. Rodriguez Del Rio P, Diaz-Perales A, Sanchez-Garcia S, Escudero C, Ibanez MD, Mendez-Brea P, et al. Profilin, a Change in the Paradigm. J Investig Allergol Clin Immunol. 2018;28(1):1-12.
20. Asero R. In patients with LTP syndrome food-specific IgE show a predictable hierarchical order. Eur Ann Allergy Clin Immunol. 2014;46(4):142-6.

21. Santos A, Van Ree R. Profilins: mimickers of allergy or relevant allergens? Int Arch Allergy Immunol. 2011;155(3):191-204.
22. Palacin A, Gomez-Casado C, Rivas LA, Aguirre J, Tordesillas L, Bartra J, et al. Graph based study of allergen cross-reactivity of plant lipid transfer proteins (LTPs) using microarray in a multicentre study. PLoS One. 2012;7(12):e50799.
23. Azofra J, Berroa F, Gastaminza G, Saiz N, Gamboa PM, Vela C, et al. Lipid Transfer Protein Syndrome in a Non-Mediterranean Area. Int Arch Allergy Immunol. 2016;169(3):181-8.
24. Pastorello EA, Farioli L, Pravettoni V, Robino AM, Scibilia J, Fortunato D, et al. Lipid transfer protein and vicilin are important walnut allergens in patients not allergic to pollen. J Allergy Clin Immunol. 2004;114(4):908-14.
25. Lauer I, Miguel-Moncin MS, Abel T, Foetisch K, Hartz C, Fortunato D, et al. Identification of a plane pollen lipid transfer protein (Pla a 3) and its immunological relation to the peach lipid-transfer protein, Pru p 3. Clin Exp Allergy. 2007;37(2):261-9.
26. Gao ZS, Yang ZW, Wu SD, Wang HY, Liu ML, Mao WL, et al. Peach allergy in China: a dominant role for mugwort pollen lipid transfer protein as a primary sensitiser. J Allergy Clin Immunol. 2013;131(1):224-6 e1-3.
27. Lombardero M, Garcia-Selles FJ, Polo F, Jimeno L, Chamorro MJ, Garcia-Casado G, et al. Prevalence of sensitisation to Artemisia allergens Art v 1, Art v 3 and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid-transfer protein allergens. Clin Exp Allergy. 2004;34(9):1415-21.
28. Wangorsch A, Jamin A, Lidholm J, Grani N, Lang C, Ballmer-Weber B, et al. Identification and implication of an allergenic PR-10 protein from walnut in birch pollen associated walnut allergy. Mol Nutr Food Res. 2017;61(4).
29. Tsakok T, Marrs T, Mohsin M, Baron S, du Toit G, Till S, et al. Does atopic dermatitis cause food allergy? A systematic review. J Allergy Clin Immunol. 2016;137(4):1071-8.
30. Villalta D, Scala E, Mistrello G, Amato S, Asero R. Evidence of Cross-Reactivity between Different Seed Storage Proteins from Hazelnut (Corylus avellana) and Walnut (Juglans regia) Using Recombinant Allergen Proteins. Int Arch Allergy Immunol. 2019;178(1):89-92.
31. Asero R, Mistrello G, Roncarolo D, Amato S, Caldironi G, Barocci F, et al. Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study. Allergy. 2002;57(10):900-6.
32. Wangorsch A, Schülke S, Gadermaier G, Albrecht M, Wallner M, Randow S, et al. LTP cross-reactivity - primary sensitisation to mugwort pollen LTP Art v 3, facilitates subsequent sensitisation to peach LTP Pru p 3 in mice . Clin Transl Allergy 2014;4.

Supplemental files

Supplemental methods on data collection

Skin Prick testing

SPT was performed with commercially available extracts (ALK-Abelló, Madrid, Spain) following guidelines of the European Academy of Allergology and Clinical Immunology.[E1]

IgE testing

IgE levels in serum were measured by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). ImmunoCAP analyses with extracts were performed at the Paul-Ehrlich-Institut (Langen, Germany). ImmunoCAP analyses with walnut components were carried out at the Amsterdam University Medical Centres (Location AMC, Amsterdam, the Netherlands).

Jug r 2 LMW

The low-molecular-weight fraction of Jug r 2 consists of the N-terminal region of Jug r 2, which is removed during maturation. It does not contain any of the mature Jug r 2 cupin domains. In the nut, the N-terminal region is found as 6 individual peptides. Here they are expressed as 1 polypeptide chain. IgE to Jug r 2 LWM was not included as a candidate predictor for prediction of severity of walnut allergy, because a considerable number of walnut allergic subjects without sensitisation to Jug r 2 were sensitised to Jug r 2 LMW at an IgE level below 1.0 kU_A/L, which in part may be due to an elevated background of this experimental assay.

Symptom severity classification

For classification of severe symptoms, *lower airway symptoms* included dyspnoea, wheezing, cough, or chest tightness; *cardiovascular symptoms* consisted of cardiac arrhythmia, myocardial ischaemia, or hypotension; *neurological symptoms* comprised disorientation/confusion, dizziness, seizures, incontinence, or loss of consciousness; and *anaphylaxis* included reactions with severe laryngeal oedema, severe bronchospasm, or hypotensive shock. For classification of mild-to-moderate symptoms, *skin symptoms* included urticaria, angioedema, erythema/flushing, or itching; *eye symptoms* comprised conjunctivitis; *upper airway symptoms* consisted of rhinitis, conjunctivitis, or tightness of throat; and *gastrointestinal symptoms* comprised stomach pain, cramps, nausea, vomiting, diarrhoea.[E2, E3]

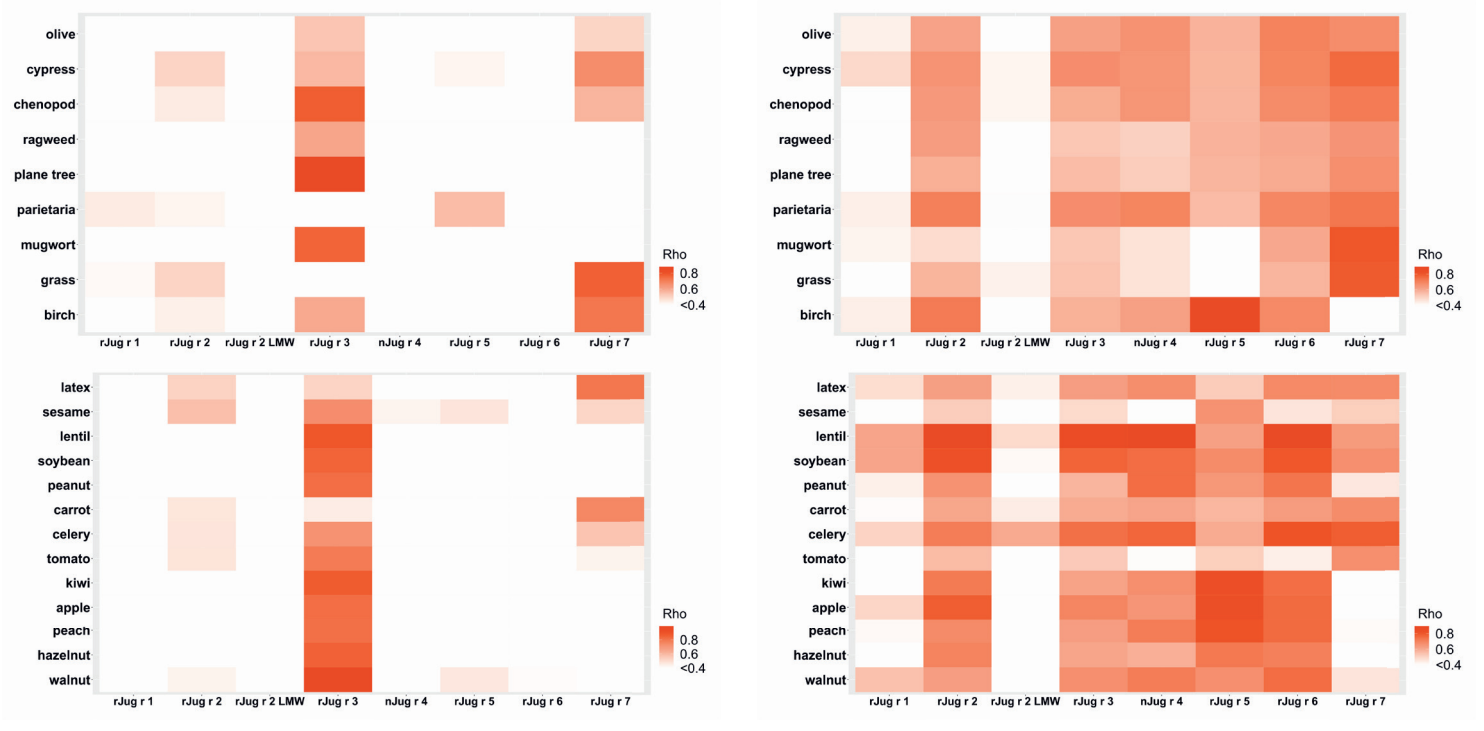

Figure S1. Correlation between IgE levels to walnut components and pollen and other foods per centre with at least 10 subjects completing CRD testing
Too few subjects completed CRD in Prague (N=8), Manchester (N=5), Reykjavik (N=3) and Sofia (N=4) to determine valid correlations. *Chenopod, Chenopodium.*

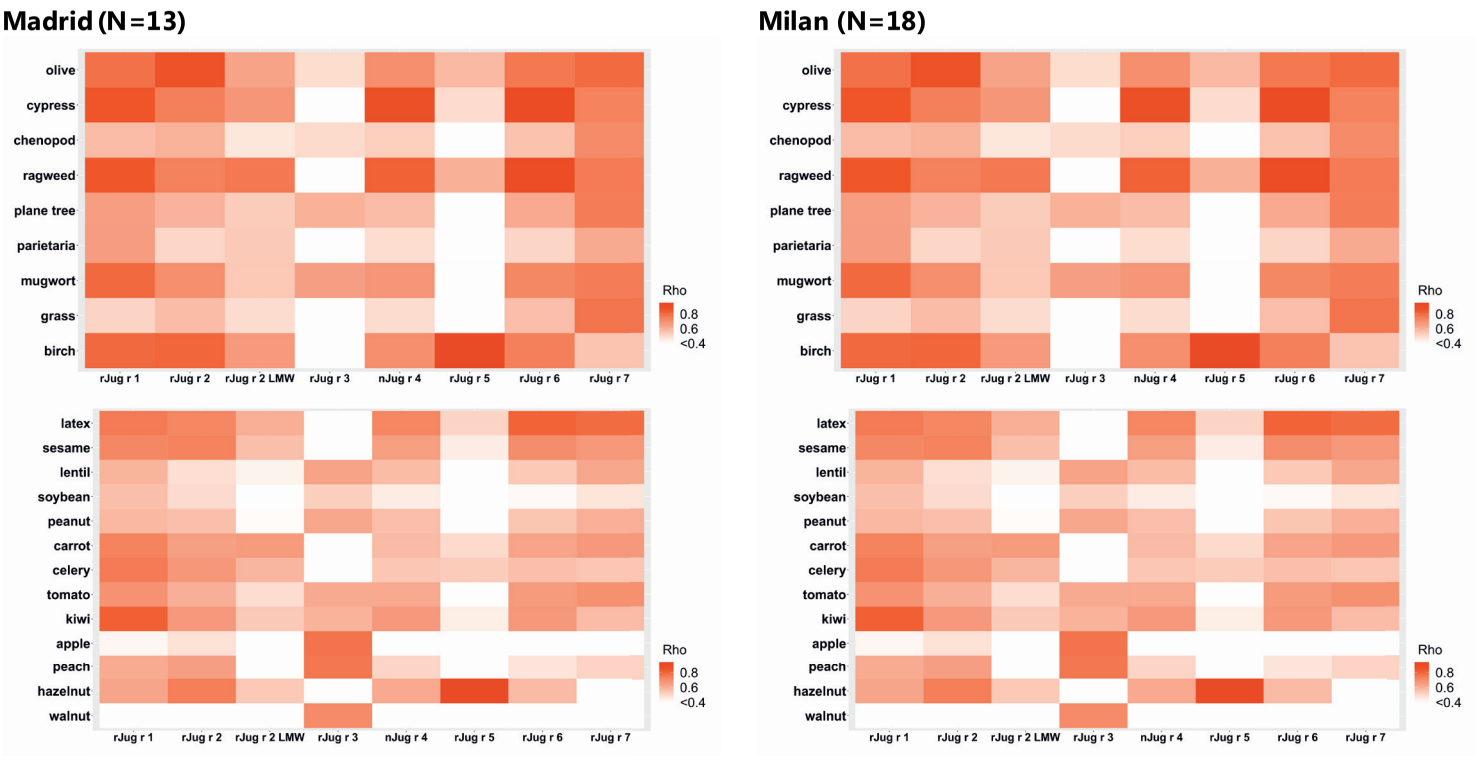

Figure S1. Correlation between IgE levels to walnut components and pollen and other foods per centre with at least 10 subjects completing CRD testing
Too few subjects completed CRD in Prague (N=8), Manchester (N=5), Reykjavik (N=3) and Sofia (N=4) to determine valid correlations. *Chenopod, Chenopodium.*

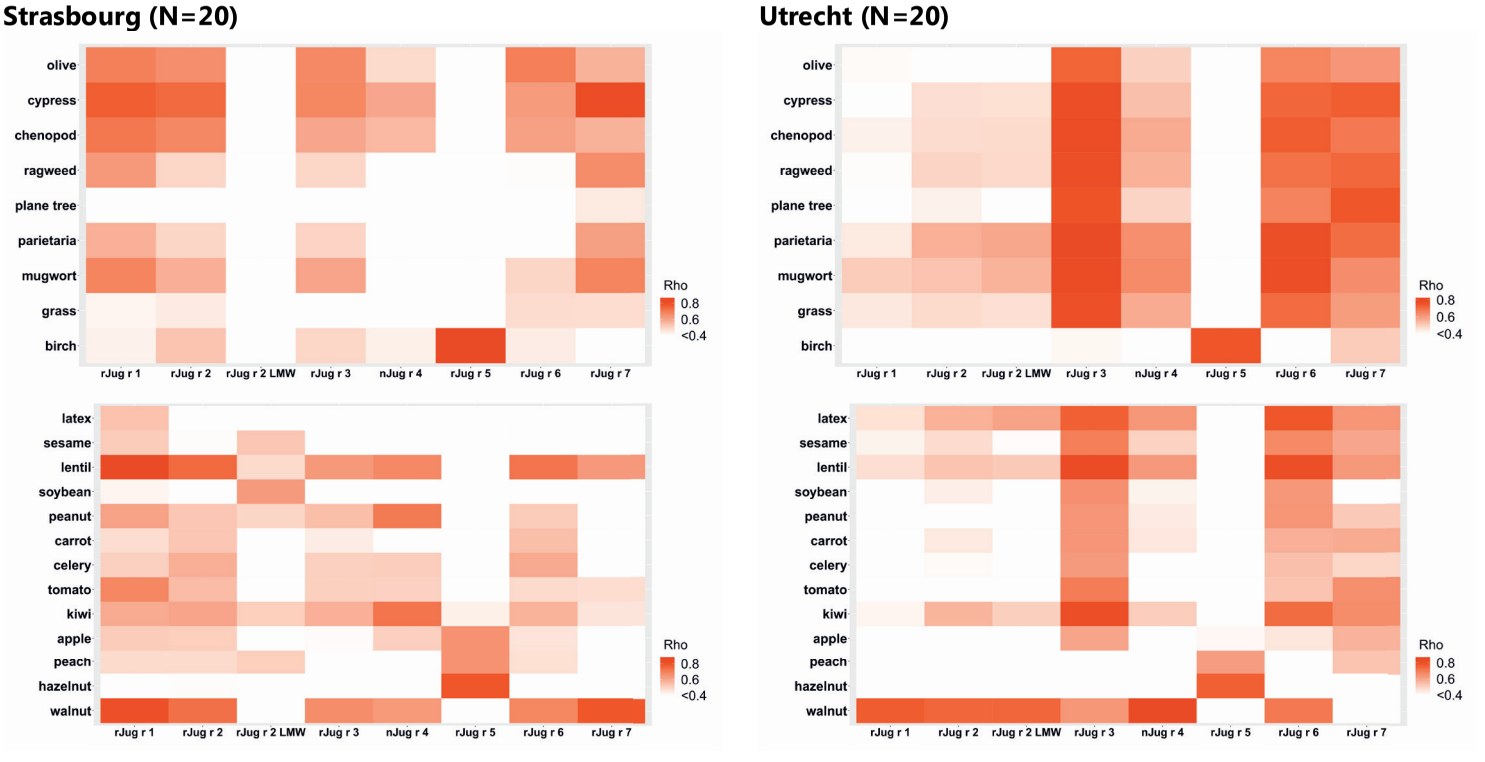

Figure S1. Correlation between IgE levels to walnut components and pollen and other foods per centre with at least 10 subjects completing CRD testing
Too few subjects completed CRD in Prague (N=8), Manchester (N=5), Reykjavik (N=3) and Sofia (N=4) to determine valid correlations. *Chenopod, Chenopodium.*

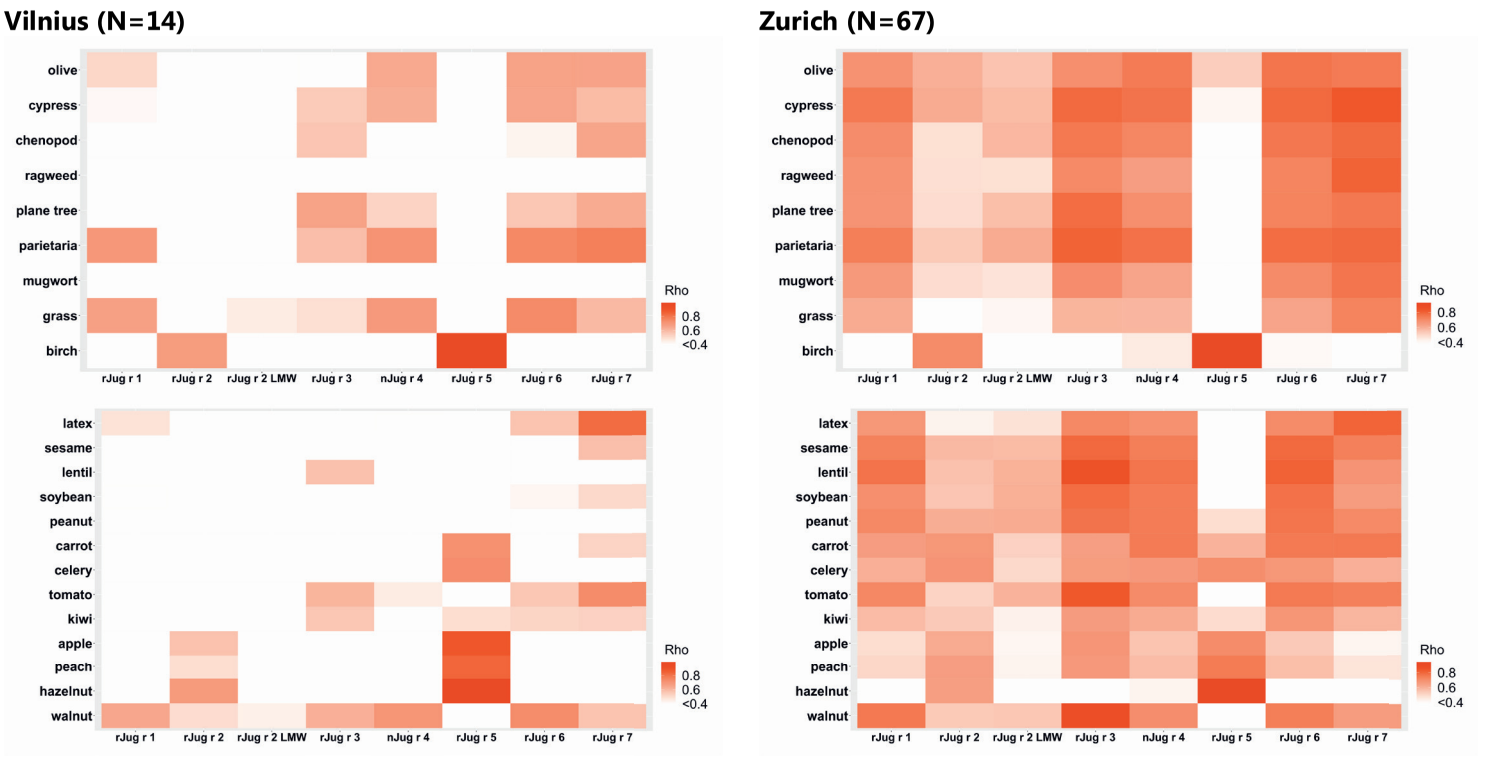

Figure S1. Correlation between IgE levels to walnut components and pollen and other foods per centre with at least 10 subjects completing CRD testing
Too few subjects completed CRD in Prague (N=8), Manchester (N=5), Reykjavik (N=3) and Sofia (N=4) to determine valid correlations. *Chenopod, Chenopodium.*

Table S1. Missing data in variables included for Lasso regression analysis

	Number of missings
Age	0
Female sex	0
Age onset symptoms	21
Symptoms upon skin contact with walnut	14
Family history of atopic disease	6
Atopic dermatitis	3
Asthma	2
Birch pollen allergy	5
Grass pollen allergy	7
Mugwort pollen allergy	4
Planetree pollen allergy	7
House dust mite allergy	6
Latex allergy	0
Cat/dog sensitisation	0
SPT walnut extract positive	0
IgE level walnut extract	0
IgE level Jug r 1	0
IgE level Jug r 2	2
IgE level Jug r 2 LMW	4
IgE level Jug r 3	0
IgE level Jug r 4	4
IgE level Jug r 5	2
IgE level Jug r 6	4
IgE level Jug r 7	0

Total N = 177. Values for the missing data were estimated using multiple imputation procedures, for which all of the above determinants were included as covariates, along with severity of walnut allergy, IgE levels to other foods (hazelnut, peach, apple, kiwi, tomato, carrot, celery, peanut, soybean, lentils, sesame seed), and centre. *SPT, skin prick test.*

Table S2. IgE to walnut components in subjects with negative walnut SPT and ImmunoCAP

	Negative ImmunoCAP		**Negative SPT**		**Negative ImmunoCAP and SPT**	
	N	**IgE level**, *median (IQR)*	**N**	**IgE level**, *median (IQR)*	**N**	**IgE level**, *median (IQR)*
Total	331	-	306	-	237	-
CRD performed	120	-	115	-	82	-
CRD positive	88/117	-	85/112	-	56 /79	-
Jug r 1	3/120	0.73 (0.69-0.80)	5/115	1.50 (0.63-3.14)	0/82	-
Jug r 2	3/117	0.75 (0.70-0.80)	5/112	0.59 (0.52-0.65)	1/79	0.65
Jug r 2 LMW	15/115	0.40 (0.37-0.50)	15/110	0.42 (0.38-0.50)	7/77	0.38 (0.36-0.41)
Jug r 3	1/120	0.89	9/115	0.89 (0.55-1.41)	1/82	0.89
Jug r 4	1/115	0.79	6/110	0.66 (0.46-0.83)	1/77	0.84
Jug r 5	79/117	11.46 (5.20-23.46)	67/112	10.75 (5.76-20.57)	50/79	9.44 (4.90-19.13)
Jug r 6	1/115	0.91	4/110	0.52 (0.41-0.73)	0/77	-
Jug r 7	21/120	1.62 (0.72-4.02)	29/115	3.91 (1.31-6.39)	16/82	1.91 (0.72-5.01)

IgE levels were measured on ImmunoCAP in kU_A/L. *CRD, component-resolved diagnostics; IQR, interquartile range; SPT, skin prick test.*

Table S3. Correlations between IgE levels to walnut components and pollen and other foods

	Jug r 1	Jug r 2	Jug r 2 LMW	Jug r 3	Jug r 4	Jug r 5	Jug r 6	Jug r 7
Birch	0.33	0.60	*0.18*	*0.22*	0.35	0.92	0.40	0.39
Grass	0.57	0.43	0.32	0.42	0.54	0.27	0.61	0.70
Mugwort	0.50	0.38	0.33	0.64	0.48	*0.21*	0.55	0.61
Parietaria	0.58	0.37	0.41	0.65	0.54	*0.19*	0.60	0.70
Plane tree	0.48	0.32	0.34	0.71	0.45	*0.18*	0.53	0.65
Ragweed	0.51	0.36	0.31	0.58	0.49	*0.24*	0.56	0.68
Chenopodium	0.55	0.36	0.38	0.68	0.53	*0.18*	0.60	0.72
Cypress	0.62	0.48	0.37	0.64	0.60	0.33	0.67	0.75
Olive	0.59	0.48	0.37	0.56	0.57	0.37	0.64	0.72
Latex	0.57	0.42	0.41	0.53	0.57	*0.20*	0.62	0.73
Sesame seed	0.61	0.50	0.44	0.61	0.59	0.27	0.67	0.65
Lentil	0.60	0.41	0.43	0.80	0.60	*0.14*	0.66	0.54
Soybean	0.55	0.40	0.40	0.71	0.55	*0.20*	0.61	0.53
Peanut	0.51	0.44	0.38	0.69	0.55	0.31	0.58	0.55
Carrot	0.53	0.56	0.33	0.45	0.53	0.57	0.58	0.68
Celery	0.50	0.57	0.30	0.51	0.47	0.65	0.53	0.57
Tomato	0.56	0.38	0.37	0.75	0.51	*0.20*	0.58	0.66
Kiwi	0.52	0.48	0.32	0.68	0.50	0.42	0.58	0.56
Apple	0.36	0.44	*0.21*	0.68	0.33	0.54	0.40	0.38
Peach	0.36	0.44	*0.23*	0.64	0.32	0.58	0.42	0.41
Hazelnut	0.37	0.64	*0.23*	0.28	0.41	0.88	0.43	0.29
Walnut	0.59	0.42	0.46	0.75	0.58	*0.01*	0.58	0.44

All correlations are Spearman's rho correlations. *Italics* indicate NOT statistically significant values after Bonferroni correction (p-value <0.007 for pollen and p-value <0.00025 for food/latex). For all other correlations, the p-values were smaller than the Bonferroni corrected p-values.

Table S4. Food extract IgE levels correlating strongly with walnut components

Centre	Jug r 1	Jug r 2	Jug r 2 LMW	Jug r 3	Jug r 4	Jug r 5	Jug r 6	Jug r 7
Zurich	Tomato Peanut Lentil Sesame	-	-	**Tomato** Peanut **Lentil** Soy **Sesame**	Carrot Tomato Peanut Lentil Soy Sesame	HN Peach Apple Celery	Carrot Tomato Peanut **Lentil** Soy **Sesame**	Carrot Tomato Peanut Sesame
Madrid	-	-	-	Peach	-	-	-	Carrot
Athens	-	-	-	**HN** **Peach** **Apple** **Kiwi** Tomato Celery **Peanut** **Soy** **Lentil** Sesame	-	-	-	Carrot
Utrecht				**Kiwi** Tomato **Lentil** Sesame		HN	Kiwi **Lentil**	-
Lodz	-	HN Apple Kiwi Celery **Soy** **Lentil**	-	Celery **Lentil** Soy	Peach Celery Peanut Soy **Lentil**	HN **Peach** **Apple** **Kiwi**	HN Peach Apple Kiwi **Celery** Peanut **Soy** **Lentil**	Celery
Vilnius	-	-	-	-	-	**HN** **Peach** **Apple** Celery Carrot	-	Tomato
Milan	**Kiwi** Celery Carrot Sesame	HN Sesame	-	Peach Apple	-	**HN**	Sesame	-
Strasbourg	**Lentil**	Lentil	-	-	Kiwi Peanut	HN	Lentil	

This table shows the food extracts, other than walnut, of which the IgE levels correlated strongly with IgE levels to walnut components in each centre. Only those foods with $\rho \geq 0.7$ and **$\rho \geq 0.8$** are shown. Only centres with at least 10 subjects completing CRD were evaluated.

Table S5. IgE levels related to severity of walnut allergy in subjects with positive serology

	Mild-to-moderate probable walnut allergy (N=246)			**Severe probable walnut allergy (N=90)**			**p**
	Total tested	**Total positive***	**IgE level**, *median (IQR)*	**Total tested**	**Total positive***	**IgE level**, *median (IQR)*	
Walnut extract	240	127 (52.9)	1.34 (0.73-3.84)	89	55 (61.8)	2.31 (1.02-7.77)	0.049
Jug r 1	136	14 (10.3)	3.13 (0.68-32.30)	41	7 (17.1)	4.40 (1.59-13.12)	0.765
Jug r 2	135	13 (9.6)	5.31 (0.75-13.15)	40	6 (15.0)	9.44 (2.48-29.62)	0.726
Jug r 2 LMW	134	35 (26.1)	0.46 (0.39-1.66)	39	8 (20.5)	5.97 (0.47-46.21)	0.126
Jug r 3	136	23 (16.9)	1.17 (0.56-2.05)	41	5 (12.2)	1.89 (1.06-2.65)	0.529
Jug r 4	134	14 (10.4)	1.57 (0.79-3.29)	39	4 (10.3)	6.42 (2.99-15.25)	0.167
Jug r 5	135	91 (67.4)	12.99 (6.63-27.59)	40	24 (60.0)	7.92 (2.63-27.59)	0.101
Jug r 6	134	9 (6.7)	0.91 (0.41-2.67)	39	3 (7.7)	7.88 (4.18-13.92)	0.518
Jug r 7	136	38 (27.9)	3.42 (1.07-6.97)	41	9 (22.0)	2.00 (0.55-2.68)	0.176

All values are N or N (%) unless otherwise specified. IgE levels were measured on ImmunoCAP in kU_A/L. *IgE ≥0.35 kU_A/L.. The p-value pertains to the difference in IgE levels between mild-to-moderate and severe probable walnut allergy. *IQR, interquartile range.*

Table S6. Accuracy of individual diagnostic tests and models for severity of walnut allergy

Individual test	AUC (95%-CI)	Positivity threshold		Mild-to-moderate	Severe	Sensitivity	95%-CI	Specificity	95%-CI	PPV	95%-CI	NPV	95%-CI
Walnut SPT	0.54 (0.44-0.65)	0.50	<	77	20	51.2	35.1-67.1	56.6	47.9-65.1	26.3	17.0-37.3	79.4	70.0-86.9
			≥	59	21								
Walnut ImmunoCAP	0.54 (0.43-0.64)	0.35	<	75	20	51.2	35.1-67.1	55.2	46.4-63.7	25.6	16.6-36.4	79.0	69.4-86.6
			≥	61	21								
		0.002	<	8	5	**87.8**	**73.8-95.9**	5.9	2.3-11.3	22.0	15.9-29.1	61.5	31.6-86.1
			≥	128	36								
		12.46	<	130	39	4.9	0.6-16.5	**95.6**	**90.6-98.4**	25.0	3.2-65.1	76.9	69.8-83.1
			≥	6	2								
Jug r 1	0.52 (0.41-0.62)	0.35	<	122	34	17.1	7.2-32.1	89.7	83.3-94.3	33.3	14.6-57.0	78.2	70.9-84.4
			≥	14	7								
		0.002	<	38	12	**70.7**	**54.5-83.9**	27.9	20.6-36.3	22.8	15.9-31.1	76.0	61.8-86.9
			≥	98	29								
		3.14	<	130	37	9.8	2.7-23.1	**95.6**	**90.6-98.4**	40.0	12.2-73.8	77.8	70.8-83.9
			≥	6	4								
Jug r 2	0.53 (0.43-0.64)	0.35	<	122	34	15.0	5.7-29.8	90.4	84.1-94.8	31.6	12.6-56.6	78.2	70.9-84.4
			≥	13	6								
		0.005	<	7	3	**92.5**	**79.6-98.4**	5.2	2.1-10.4	22.4	16.3-29.6	70.0	34.8-93.3
			≥	128	37								
		5.31	<	129	36	10.0	2.8-23.7	**95.6**	**90.6-98.4**	40.0	12.2-73.8	78.2	71.1-84.2
			≥	6	4								
Jug r 2 LMW	0.53 (0.42-0.63)	0.35	<	99	31	20.5	9.3-36.5	73.9	65.6-81.1	18.6	8.4-33.4	76.2	67.2-83.2
			≥	35	8								
		0.11	<	13	3	**92.3**	**79.1-98.3**	9.7	5.3-16.0	22.9	16.6-30.3	81.3	54.4-96.0
			≥	121	36								
		9.12	<	128	35	10.3	2.9-24.2	**95.5**	**90.5-98.3**	40.0	12.2-73.8	78.5	71.4-84.6
			≥	6	4								
Jug r 3	0.48 (0.38-0.58)	0.35	<	113	36	12.2	4.1-26.2	83.1	75.7-89.0	17.9	6.1-36.9	75.8	68.2-82.5
			≥	23	5								
		0.006	<	17	5	**87.8**	**73.8-95.9**	12.5	7.5-19.3	23.2	16.8-20.7	77.3	54.6-92.2
			≥	119	36								
		2.01	<	130	39	4.9	0.6-16.5	**95.6**	**90.6-98.4**	25.0	3.2-65.1	76.9	69.8-83.1
			≥	6	2								

Table S6. Accuracy of individual diagnostic tests and models for severity of walnut allergy (continued)

Individual test	AUC (95%-CI)	Positivity threshold		Mild-to-moderate	Severe	Sensitivity	95%-CI	Specificity	95%-CI	PPV	95%-CI	NPV	95%-CI
Jug r 4	0.57 (0.46-0.68)	0.35	<	120	35	10.3	2.9-24.2	89.6	83.1-94.2	22.2	6.4-47.6	77.4	70.0-83.7
			≥	14	4								
		0.003	<	4	3	**92.3**	**79.1-98.4**	3.0	0.8-7.5	21.7	15.7-28.7	57.1	18.4-90.1
			≥	130	36								
		2.07	<	128	36	7.7	1.6-20.9	**95.5**	**90.5-98.3**	33.3	7.5-70.2	78.0	70.9-84.1
			≥	6	3								
Jug r 5	0.58 (0.49-0.68)	0.35	<	44	16	60.0	43.3-75.1	32.6	24.8-41.2	20.9	13.9-29.4	73.3	60.3-83.9
			≥	91	24								
		0.003	<	6	3	**92.5**	**79.6-98.4**	4.4	1.7-9.4	22.3	16.2-29.4	66.7	29.9-92.5
			≥	129	37								
		62.73	<	129	40	0.0	0.0-8.8	**95.6**	**90.6-98.4**	0.0	0.0-45.9	76.3	69.2-82.5
			≥	6	0								
Jug r 6	0.54 (0.44-0.65)	0.35	<	125	36	7.7	1.6-20.9	93.3	87.6-96.9	25.0	5.5-57.2	77.6	70.4-83.8
			≥	9	3								
		0.005	<	8	3	**92.3**	**79.1-98.4**	6.0	2.6-11.4	22.2	16.1-29.4	72.7	39.0-94.0
			≥	126	36								
		0.41	<	128	36	7.7	1.6-20.7	**95.5**	**90.5-98.3**	33.3	7.5-70.1	78.0	70.9-84.1
			≥	6	3								
Jug r 7	0.53 (0.44-0.63)	0.35	<	98	32	22.0	10.6-37.6	72.1	63.7-79.4	19.1	9.2-33.3	75.4	67.1-82.5
			≥	38	9								
		0.004	<	42	12	**70.7**	**54.5-83.9**	30.9	23.2-39.4	23.6	16.4-32.1	77.8	64.4-88.0
			≥	94	29								
		15.00	<	130	41	0.0	0.0-8.6	**95.6**	**90.6-98.4**	0.0	0.00-45.9	76.0	68.9-82.2
			≥	6	0								
Model	**AUC (95%-CI)**	**Positivity threshold**		**Mild-to-moderate**	**Severe**	**Sensitivity**	**95%-CI**	**Specificity**	**95%-CI**	**PPV**	**95%-CI**	**NPV**	**95%-CI**
Model CRD only*	0.66 (0.57-0.75)	0.50	<	128	35	5.4	0.7-18.2	100.0	97.2-100.0	100.0	15.8-100	78.5	71.4-84.6
			≥	0	2								
		0.16	<	33	3	**91.9**	**78.1-98.3**	25.8	18.5-34.3	26.4	19.0-34.8	91.7	77.5-98.3
			≥	95	34								
		0.30	<	123	33	10.8	3.0-25.4	**96.1**	**91.1-98.7**	44.4	13.7-78.8	78.9	71.6-85.0
			≥	5	4								

Table S6. Accuracy of individual diagnostic tests and models for severity of walnut allergy (continued)

Model	AUC (95%-CI)	Positivity threshold		Mild-to-moderate	Severe	Sensitivity	95%-CI	Specificity	95%-CI	PPV	95%-CI	NPV	95%-CI
Model I	0.74 (0.65-0.83)	0.50	<	103	29	9.4	2.0-25.0	100.0	96.5-100.0	100.0	29.2-100	78.0	70.0-84.8
			≥	0	3								
		0.17	<	56	8	**75.0**	**56.6-88.5**	54.4	44.3-64.2	33.8	23.0-46.1	87.5	76.9-94.5
			≥	47	24								
		0.34	<	99	23	28.1	13.8-46.8	**96.1**	**90.4-98.9**	69.2	38.6-90.9	81.2	73.1-87.7
			≥	4	9								
Model II	0.74 (0.65-0.83)	0.50	<	102	25	21.9	9.3-40.0	99.0	94.7-100.0	87.5	47.4-99.7	79.5	71.5-96.8
			≥	1	7								
		0.14	<	56	7	**78.1**	**60.0-90.7**	54.4	44.3-64.2	34.7	23.9-46.9	88.9	78.4-95.4
			≥	47	25								
		0.36	<	99	23	28.1	13.8-46.8	**96.1**	**90.4-98.9**	69.2	38.6-90.9	81.2	73.1-87.7
			≥	4	9								
Model III	0.81 (0.73-0.89)	0.50	<	97	23	23.3	9.9-42.3	98.0	92.9-99.8	77.8	40.0-97.2	80.8	72.6-87.4
			≥	2	7								
		0.14	<	39	3	**90.0**	**73.5-97.9**	39.4	29.7-49.7	31.0	21.6-41.9	92.9	80.5-98.5
			≥	60	27								
		0.39	<	95	14	53.3	34.3-71.7	**96.0**	**90.0-99.9**	80.0	56.3-94.8	87.2	79.4-92.8
			≥	4	16								

Measures of accuracy were calculated for each of the individual diagnostic tests, and for the models on clinical background variables (model I), clinical background variables + sensitisation to walnut extract in SPT or ImmunoCAP (model II), and clinical background variables + sensitisation to walnut extract + sensitisation to walnut components (model III). The three rows of threshold values given for each diagnostic test respectively indicate the cutoffs generally used in clinical practice, corresponding with a high sensitivity (closest to 95%), and corresponding with a high specificity (closest to 95%). **Bold** indicates the sensitivity and specificity estimates closest to 95%. *Model including Jug r 1, 2, 3, 4, 5, 6, 7 and Ana c 2 (not Jug r 2 LMW). *CI, confidence interval; CRD, component-resolved diagnostics; NPV, negative predictive value; PPV, positive predictive value; SPT, skin prick test.*

References supplemental files

E1. Position paper: Allergen standardization and skin tests. The European Academy of Allergology and Clinical Immunology. Allergy. 1993;48(14 Suppl):48-82.
E2. Muraro A, Fernandez-Rivas M, Beyer K, Cardona V, Clark A, Eller E, et al. The urgent need for a harmonized severity scoring system for acute allergic reactions. Allergy. 2018;73(9):1792-800.
E3. Sampson HA. Anaphylaxis and emergency treatment. Pediatrics. 2003;111(6 Pt 3):1601-8.

Chapter 8

Estimating the risk of severe peanut allergy using clinical background and IgE sensitisation profiles

Manuscript submitted

Sarah A. Lyons,* Mareen R. Datema,* Montserrat Fernández-Rivas, Barbara Ballmer-Weber, André C. Knulst, Riccardo Asero, Laura Barreales, Simona Belohlavkova, Frédéric de Blay, Michael Clausen, Ruta Dubakiene, Cristina Fernández-Pérez, Philipp Fritsche, David Gislason, Karin Hoffmann-Sommergruber, Monika Jedrzejczak-Czechowicz, Laurian Jongejan, Marek Kowalski, Tanya Z. Kralimarkova, Jonas Lidholm, Nikolaos G. Papadopoulos, Todor A. Popov, Nayade del Prado, Ashok Purohit, Isabel Reig, Suranjith L. Seneviratne, Athanassios Sinaniotis, Serge A. Versteeg, Stefan Vieths, Paco M.J. Welsing, E.N. Clare Mills, Thuy-My Le, Aeilko H. Zwinderman, Ronald van Ree

*Contributed equally

Abstract

Background
It is not well understood why symptom severity varies between patients with peanut allergy (PA).

Objective
To gain insight into the clinical profiles of subjects with mild-to-moderate and severe PA, and investigate individual and collective predictive accuracy of clinical background, IgE to peanut extract and IgE to peanut components for PA severity.

Methods
Data on demographics, patient history, and sensitisation at extract and component level of 393 patients with probable PA (symptoms ≤2 hours + IgE sensitisation) from 12 EuroPrevall centres were analysed. Univariable and penalised multivariable regression analyses were used to evaluate risk factors and biomarkers for severity.

Results
Female sex, age at onset of PA, symptoms elicited by skin contact with peanut, family atopy, atopic dermatitis, house dust mite allergy and latex allergy were independently associated with severe PA; birch pollen allergy with mild-to-moderate PA. The cross-validated AUC of all clinical background determinants combined (0.74) was significantly larger than the AUC of tests for sensitisation to extract (0.63) or peanut components (0.54-0.64). Although larger skin prick test wheal size, and higher IgE to peanut extract, Ara h 1 and Ara h 2/6, were associated with severe PA, and higher IgE to Ara h 8 with mild-to-moderate PA, addition of these measurements of sensitisation to the clinical background model did not significantly improve the AUC.

Conclusions
Models combining clinical characteristics and IgE sensitisation patterns can help establish the risk of severe reactions for peanut allergic patients, but clinical background determinants are most valuable for predicting severity of probable PA in an individual patient.

Introduction

Patients with peanut allergy (PA) often require strict elimination diets to prevent potentially severe allergic reactions. Beyond levels of exposure, it is not well understood why symptom severity varies between patients.[1]

To gain insight into severity of PA in a particular patient, accurate clinical evaluation is essential. Besides patient history, routine diagnostic tests include extract-based skin prick testing (SPT) and serum IgE measurements. There is conflicting evidence on the usefulness of SPT and IgE levels for predicting severity of PA.[2-5] In recent years, serum IgE testing using whole food extracts has been complemented with allergen component testing. For peanut, IgE to Ara h 2 has been demonstrated to better distinguish PA from tolerance than IgE to peanut extract.[6-14] Some studies have reported a relationship between IgE levels to Ara h 2 and severity of PA,[7, 11, 14-16] whereas other studies report no clear difference.[6, 12, 17, 18] Food challenge, preferably double-blind placebo-controlled food challenge (DBPCFC), is the reference standard for confirming presence and severity of PA. However, due to the burdensome and resource-intensive nature of food challenge, daily practice diagnosis is often based on a suggestive patient history in combination with IgE sensitisation (i.e. probable PA).[19]

Peanut and tree nuts are reportedly the most common causes of food-induced anaphylaxis.[1] In recent papers on hazelnut allergy[20] and walnut allergy[21], we set out to develop prediction models in which a patient's demographic and clinical background is combined with results from routine extract-based tests and from component-resolved diagnostics (CRD). For both tree nuts, models combining clinical background information with measures of IgE sensitisation were shown to improve the accuracy of predicting severe reactions significantly compared with clinical variables, IgE to extract, or IgE to allergen components alone. Although several previous studies have evaluated the predictive accuracy of combined clinical and serological information for predicting PA,[6, 7, 22, 23] the focus is rarely on prediction of *severity*. Petterson *et al*. developed a model for severe PA based on clinical characteristics and serum IgE to peanut extract, but did not assess contribution of CRD, and included only children.[22]

In the present study, we evaluated data collected from predominantly adult patients reporting PA during the EuroPrevall outpatient clinic surveys in 12 different European cities,[16] using an approach comparable to that in previous evaluations for hazelnut and walnut. In a subset of these patients who underwent DBPCFC, Ballmer-Weber and colleagues previously reported that systemic reactions occurred significantly more frequently in subjects sensitised to peanut extract (IgE ≥0.35 kU/L) or to Ara h 2 (IgE ≥1.0 kU/L).[16] Our aim was to further investigate the

association of demographics, clinical background, and markers of peanut sensitisation, with the severity of PA, and to subsequently develop prediction models using all this information to improve discriminatory ability for estimating the risk of severe reactions.

Methods

Study design and population

Twelve European allergy centres in Athens (Greece), Lodz (Poland), Madrid (Spain), Manchester (United Kingdom), Milan (Italy), Prague (Czech Republic), Reykjavik (Iceland), Sofia, (Bulgaria), Strasbourg (France), Utrecht (the Netherlands), Vilnius (Lithuania) and Zurich (Switzerland), enrolled patients with a history of food allergy (FA) in the EuroPrevall outpatient clinic study. Each local ethical committee approved the study. Recruitment took place between 2006 and 2009. Informed consent was documented for all patients before enrolment in the study. For the current study, we included all patients reporting adverse reactions within 2 hours of ingestion of peanut.

Clinical evaluation

The methodology of the EuroPrevall outpatients study has been described in detail elsewhere.[24] All patients underwent a physician-administered questionnaire focusing on reaction characteristics and allergic comorbidities. Skin prick test (SPT) reactivity to peanut extract was assessed using a commercially available extract (ALK-Abelló, Madrid, Spain). Serum samples were collected locally in each centre, and analysed by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) at the Paul-Ehrlich Institute (Langen, Germany). All available sera were tested for sensitisation to peanut extract, as well as to other food and inhalant allergens.[24] A custom-made microarray chip was used to test for sensitisation to food allergen components, amongst which were peanut allergens nAra h 1 (7S globulin), nAra h 2/6 (2S albumin), nAra h 3 (11S globulin), and rAra h 8 (Pathogenesis-related protein family 10 [PR-10] protein).[25] DBPCFC was carried out in all consenting subjects by trained clinicians as described previously.[26] Patients were excluded from DBPCFC if they had a history of severe life-threatening anaphylaxis to peanut, involving hypotension, severe bronchospasm or laryngeal oedema within 2 hours of ingestion, leading to emergency treatment.[24]

Definitions

Patients who, along with symptoms within 2 hours of peanut ingestion, had IgE sensitisation to peanut, as measured by positive SPT, ImmunoCAP or microarray, were defined as having *probable PA*. SPT allergen/histamine wheal ratios were considered positive at a ratio ≥0.5, IgE in ImmunoCAP at levels ≥0.35 kU_A/L, and IgE in microarray at levels ≥0.3 ISU/L.

Severity of symptoms was classified into 2 groups: *Mild-to-moderate* if isolated oral allergy, upper airway, skin and/or gastrointestinal symptoms occurred; *severe* in case of symptoms of the lower airway (either laryngeal or bronchial), cardiovascular or neurological system.[27, 28]

Patients with proven sensitisation in SPT or ImmunoCAP matching their reported rhinoconjunctivitis or asthma symptoms to birch, grass, mugwort, house dust mite (HDM) or latex were considered to be allergic to the respective allergen sources.

Statistical analysis

All analyses were performed in subjects with probable PA. In univariable analysis, differences in demographic factors and clinical background (age, sex, age at onset of PA [<14 years *versus* ≥14 years], symptoms upon skin contact with peanuts, first degree family members with atopy, atopic dermatitis [ever], allergy to pollen, HDM or latex, and sensitisation to cats or dogs), results from extract-based testing (SPT and ImmunoCAP with peanut extract), and results from CRD (microarray Ara h 1, 2/6, 3 and 8), were evaluated using chi-square tests, independent sample t-tests, or Mann-Whitney U tests where appropriate. Bonferroni corrections were used to correct for multiple testing.

Multivariable analyses were performed to identify the most relevant set of predictors for severity of probable PA. To limit overfitting and improve generalisability, the Least Absolute Shrinkage and Selection Operator (Lasso) regression approach was chosen. This method selects only the most discriminative combination of variables, and applies cross-validation to shrink regression coefficients.[29] To ensure use of all data, missing data were imputed ten-fold using multi-chain Monte Carlo methods with the mice package in R. Details on missing data and included covariates are available from table S1. Lasso regression was repeated on each of the 10 imputed datasets. Predictor variables selected in at least 7 of the 10 imputed datasets were included. Bootstrapping was used to estimate 95% confidence intervals (CI) for each coefficient. Results were pooled using Rubin's rules.

A stepwise approach to model building was taken, and the Lasso selection process was applied in each step. In model I, all variables on demographics and clinical background were entered. In model II, peanut extract-based test results (SPT [wheal ratios] and ImmunoCAP [IgE levels]) were added to the selected model I variables. In model III, peanut CRD results were entered, along with the variables selected in model II. Finally, to explore if knowledge of IgE levels to plant source food extracts and components other than peanut could improve prediction of PA severity, ImmunoCAP and CRD results related to sensitisation to soybean, lentil, hazelnut, walnut, sesame seed, peach, apple, kiwi, tomato, carrot, and celery, were entered in

a final step, after fixing the variables selected in model III. The discriminatory ability of the resulting regression models to distinguish between mild-to-moderate and severe probable PA was quantified by area under the receiving operating curve (AUC) estimators. AUCs were compared using DeLong's test.[30]

For comparative purposes, Lasso regression analyses were repeated in the subgroup of subjects with clinically determined symptom severity based on DBPCFC or a convincing history of severe anaphylaxis. Subjects with a negative DBPCFC outcome and placebo reactors were grouped with the mild-to-moderate DBPCFC reactors.

Analyses were conducted with R version 3.4.1.

Results

Of the 517 subjects reporting symptoms within 2 hours of ingestion of peanut, 393 (76%) had probable PA. Overall, 216 (55%) had mild-to-moderate and 177 (45%) had severe probable PA (Table 1, Figure S1). Of the subjects with mild-to-moderate probable PA, 89/216 (41%) had isolated oral allergy symptoms (OAS).

Demographic and clinical characteristics associated with severity of probable PA

Frequencies of demographic and clinical background characteristics of patients with mild-to-moderate and those with severe probable PA are presented in Table 1 and Figure 1. Subjects with a severe phenotype were younger than those with the mild-to-moderate phenotype, and manifestation of probable PA more often occurred before the age of 14 years. Subjects with a severe phenotype were more likely to have symptoms elicited by skin contact with peanut, atopic dermatitis, HDM allergy, latex allergy or sensitisation to cats and/or dogs, but less likely to be allergic to birch pollen.

Measures of IgE sensitisation associated with severity of probable PA

Of subjects with probable PA, 320/387 (83%) had a positive SPT and 284/376 (76%) had a positive ImmunoCAP test to peanut extract (Table 1), and 240/370 (65%) tested positive to both tests. The allergen/histamine wheal ratios and levels of IgE to peanut extract were significantly higher in patients with severe symptoms than in patients with mild-to-moderate symptoms (Table 1 and Figure 1).

Table 1. Characteristics of subjects with probable peanut allergy

	Mild-to-moderate (N=216)	Severe (N=177)	p
Demographics			
Age at visit in years, *mean (±SD)*	28.2 (±14.3)	24.8 (±13.7)	**0.019**
Age <14 years	30/216 (13.9)	39/177 (22.0)	**0.048**
Female sex	126/216 (58.3)	106/177 (59.9)	0.835
Clinical background			
Age at onset of symptoms < 14 years	86/211 (40.8)	113/174 (64.9)	**<0.001***
Symptoms upon skin contact with peanut	10/192 (5.2)	48/146 (32.9)	**<0.001***
Family history of atopic disease	131/210 (62.4)	123/176 (69.9)	0.150
Atopic dermatitis	62/212 (29.2)	89/175 (50.9)	**<0.001***
Birch pollen allergy[‡]	124/213 (58.2)	81/172 (47.1)	**0.038**
Grass pollen allergy[‡]	124/213 (58.2)	109/172 (63.4)	0.355
Mugwort pollen allergy[‡]	42/213 (19.7)	23/172 (13.4)	0.130
House dust mite allergy[‡]	98/201 (48.8)	106/160 (66.2)	**0.001**
Latex allergy[‡]	10/195 (5.1)	23/165 (13.9)	**0.007**
Cat/dog sensitisation [‡]	146/215 (67.9)	137/175 (78.3)	**0.030**
Peanut sensitisation[§]			
SPT peanut extract			
Positive	176/212 (83.0)	144/175 (82.3)	0.956
Allergen/histamine wheal ratio, *median (IQR)*	0.78 (0.57-1.00)	1.07 (0.64-1.80)	**<0.001***
ImmunoCAP peanut extract			
Positive	144/209 (68.9)	140/167 (83.8)	**0.001***
IgE level, *median (IQR)*	0.95 (0.22-3.23)	2.21 (0.75-12.84)	**<0.001***
Microarray peanut allergens[**]			
Ara h 1			
Positive	26/176 (14.8)	54/144 (37.5)	**<0.001***
IgE level, *median (IQR)*	0.00 (0.00-0.00)	0.00 (0.00-0.83)	**0.004**
Ara h 2/6			
Positive	19/176 (10.8)	56/144 (38.9)	**<0.001***
IgE level, *median (IQR)*	0.00 (0.00-0.00)	0.00 (0.00-6.89)	**<0.001***
Ara h 3/3.02			
Positive	10/176 (5.7)	43/144 (29.9)	**<0.001***
IgE level, *median (IQR)*	0.00 (0.00-0.00)	0.00 (0.00-0.49)	**0.001**
Ara h 8			
Positive	112/176 (63.6)	67/144 (46.5)	**0.003**
IgE level, *median (IQR)*	0.44 (0.00-1.21)	0.12 (0.00-0.82)	0.096

All measurements are in n/N (%) unless otherwise specified. P-values indicate difference between patients with mild-to-moderate and patients with severe allergic symptoms to peanut. **Bold** indicates p<0.05. *Differences remained significant after Bonferroni correction. [‡]Reported symptoms + matching sensitisation by SPT or ImmunoCAP. [§]Not all patients had complete testing for peanut sensitisation. [**]Allergen components measured by microarray in 322 patients. *IQR, interquartile range; SPT, skin prick test.*

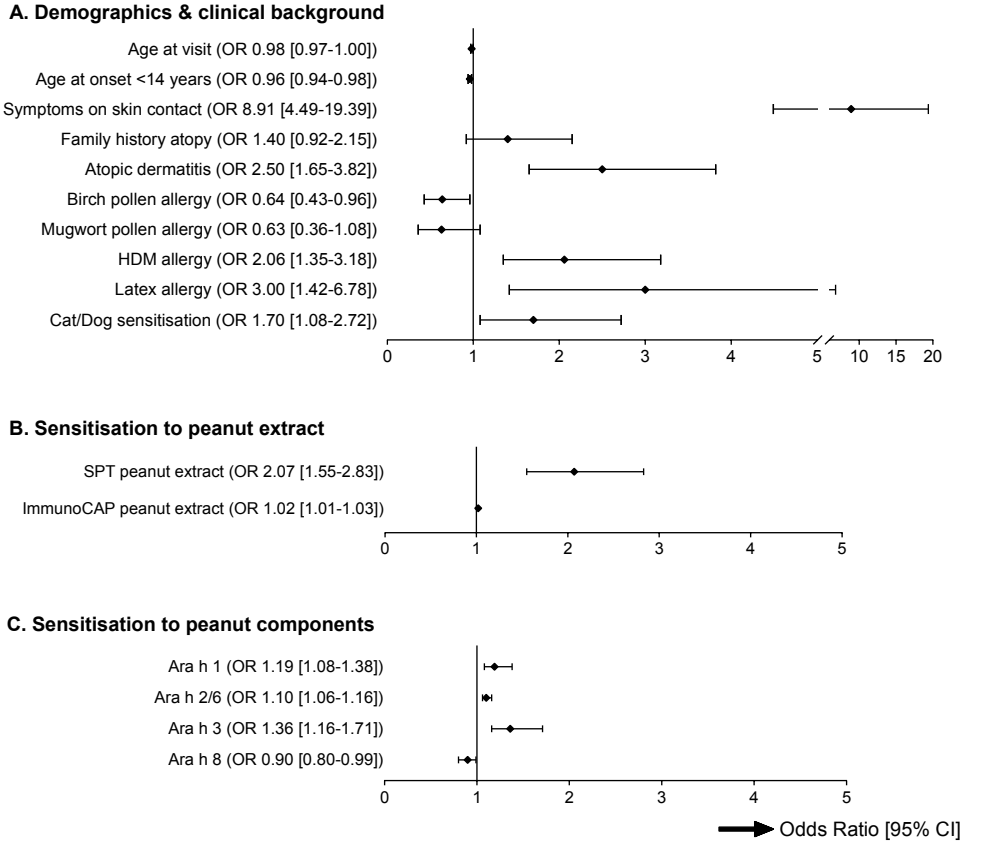

Figure 1. Univariable odds ratios for prediction of severity of probable PA ($p<0.2$)
This forest plot shows the ORs and their respective confidence intervals from univariable analyses of all predictors for severity of probable peanut allergy with $p<0.2$ (Table 1). All variables under B and C, and 'age at visit' were entered as continuous variables. All other variables were dichotomous.

Microarray was performed in 322 of 391 (82%) subjects with probable PA, and 230/322 (71%) were sensitised to at least one peanut component. All 27 component-sensitised subjects who were not sensitised to peanut extract in SPT or ImmunoCAP, were sensitised to Ara h 8 (Table S2). Overall, sensitisation to Ara h 8 was most common, and associated with mild-to-moderate probable PA (although not significantly after Bonferroni correction). Sensitisation to Ara h 1, Ara h 2/6 or Ara h 3 was associated with severe probable PA, and IgE levels to these components were significantly higher in those with severe symptoms (Table 1 and Figure 1).

Regarding foods other than peanut, IgE levels to extract from other legumes, soybean and lentil, were higher in subjects with severe probable PA than in those with mild-to-moderate probable PA (Table S3). At a molecular level, subjects with severe probable PA were significantly more often sensitised to soybean Gly m 5 (7S

globulin) and Gly m 6 (11S globulin), hazelnut Cor a 11 (7S globulin), walnut Jug r 2 (7S globulin), and sesame Ses i 1 (2S albumin) (Table S4). IgE levels to peach, apple and celery extract were higher in subjects with mild-to-moderate probable PA than in subjects with severe probable PA. The mild-to-moderately peanut allergic subjects were more often sensitised to PR-10 proteins Gly m 4 (soybean), Cor a 1 (hazelnut), and Mal d 1 (apple).

Discriminating between mild-to-moderate and severe probable PA

The AUCs of single tests (SPT peanut extract, ImmunoCAP peanut extract, microarray peanut components) for discriminating between patients with mild-to-moderate and severe probable PA ranged from 0.54 to 0.64 (Table S5). The accuracy of SPT wheal ratio and of peanut extract and component IgE levels at specific cutoffs, are shown in supplementary table S6. The most discriminative model combining microarray results comprised IgE levels to Ara h 2/6 and Ara h 8, with an AUC of 0.65 (95%-CI 0.63-0.66). The AUCs of our 3 models taking demographic and clinical factors as starting point, and combining those with markers for peanut extract and component sensitisation, were significantly larger than the AUCs of the single peanut sensitisation tests ($P_{De\ Long's\ test}$ <0.001) (Table 2 and E5).

Table 2. Prediction models for severity of probable PA

	Model I: Demographics & clinical background		**Model II:** Model I + sensitisation to peanut extract		**Model III:** Model II + sensitisation to peanut components	
	OR	**95%-CI**	**OR**	**95%-CI**	**OR**	**95%-CI**
Age at onset <14 years	1.34	0.84-2.13	1.16	0.77-1.77	1.15	0.77-1.70
Female sex	1.27	0.82-1.97	1.30	0.83-2.04	1.29	0.84-1.99
Family atopy	1.35	0.85-2.15	1.35	0.85-2.16	1.31	0.85-2.01
Atopic dermatitis	1.51	0.93-2.44	1.43	0.90-2.27	1.46	0.91-2.35
Symptoms skin contact	5.71	2.98-10.93	4.78	2.47-9.25	4.57	2.33-8.89
Birch pollen allergy	0.61	0.37-1.01	0.63	0.38-1.04	0.57	0.44-1.15
HDM allergy	1.58	0.98-2.56	1.47	0.91-2.36	1.43	0.91-2.25
Latex allergy	1.71	0.73-4.00	1.73	0.78-3.86	1.67	0.74-1.58
SPT peanut extract			1.26	0.98-1.61	1.22	0.94-1.58
IgE level peanut extract			1.01	1.00-1.01	1.00	1.00-1.01
IgE level Ara h 1					1.02	0.95-1.05
IgE level Ara h 2/6					1.01	0.98-1.04
IgE level Ara h 8					0.95	0.87-1.03
Intercept	*-1.25*		*-1.40*		*-1.36*	
AUC (95%-CI)	0.74 (0.72-0.75)		0.74 (0.73-0.76)		0.75 (0.74-0.77)	

The area under the curve (AUC) indicates the ability of the model to discriminate between patients with mild-to-moderate and patients with severe allergic symptoms to peanuts. *HDM, house dust mite; SPT, skin prick test.*

In the first model, female sex, age at onset of PA <14 years, symptoms elicited by skin contact with peanut, family atopy, atopic dermatitis, birch pollen allergy, HDM allergy, and latex allergy, were selected by Lasso regression. All determinants, except for birch pollen allergy, were associated with severe probable PA. This combination of clinical and demographic factors resulted in an AUC of 0.74 (95%-CI 0.72-0.75). Lasso regression selected SPT wheal size ratio and ImmunoCAP IgE level to peanut extract (both associated with severe PA) as additionally contributing variables in model II, and IgE to Ara h 1 and Ara h 2/6 (severe) and Ara h 8 (mild-to-moderate) in model III, although the AUC showed only a limited increase (Table 2). After model III, no IgE levels to foods and food components other than peanut were additionally selected to help discriminate between mild-to-moderate and severe PA.

Discriminating between mild-to-moderate and severe symptoms to peanut in subjects who underwent DBPCFC, or experienced severe anaphylaxis

A total of 52/393 subjects with probable PA agreed to undergo DBPCFC, of which 23 were positive, 18 were negative, 7 were placebo reactive, and 4 were excluded from analyses because of incomplete data. Severe anaphylaxis was determined in 43/393 subjects with probable PA based on patient history. Details on demographics, clinical variables, SPT and IgE results of subjects with no or mild-to-moderate symptoms during DBPCFC (N=47) and of subjects with severe symptoms during DBPCFC (N=1) or convincing history of severe anaphylaxis (N=43), are available from table S7.

Table 3. Prediction models for severity of PA according to DBPCFC or history of anaphylaxis

	Model I: Demographics & clinical background		**Model II:** Model I + sensitisation to peanut extract		**Model III:** Model II + sensitisation to peanut components	
	OR	**95%-CI**	**OR**	**95%-CI**	**OR**	**95%-CI**
Age at visit	0.95	0.90-1.01	0.96	0.91-1.02	0.96	0.90-1.03
Female sex	2.37	0.69-8.14	2.43	0.62-9.57	2.64	0.34-20.77
Family atopy	5.53	1.45-21.06	4.97	1.27-19.45	5.16	1.15-23.14
Symptoms skin contact	9.93	2.22-44.39	9.00	1.83-44.33	8.69	0.97-77.97
Birch pollen allergy	0.64	0.19-2.14	0.61	0.18-2.14	0.57	0.12-2.65
Grass pollen allergy	0.39	0.09-1.63	0.40	0.09-1.76	0.43	0.08-2.28
HDM allergy	3.11	0.75-12.84	2.96	0.67-12.99	2.85	0.64-12.59
IgE level peanut extract			1.01	0.99-1.03		
IgE level Ara h 1					1.08	0.71-1.63
IgE level Ara h 8					1.06	0.75-1.48
Intercept	*-1.33*		*-1.60*		*-1.74*	
AUC (95%-CI)	0.74 (0.72-0.75)		0.74 (0.73-0.76)		0.75 (0.74-0.77)	

The area under the curve (AUC) indicates the ability of the model to discriminate between patients with mild-to-moderate and patients with severe allergic symptoms to peanuts. *HDM, house dust mite; SPT, skin prick test.*

Just like for probable PA, symptoms elicited by skin contact with peanut (associated with severe PA), female sex (severe), family atopy (severe), birch pollen allergy (mild-to-moderate) and HDM allergy (severe) were selected as demographic and clinical background predictors for PA in the DBPCFC/anaphylaxis subgroup, with additionally lower age at visit (mild-to-moderate) and grass pollen allergy (mild-to-moderate). IgE to peanut extract (severe) was selected in model II, but no longer in model III, where IgE to Ara h 1 (severe) and Ara h 8 (severe) were favoured. The AUC of these models ranged from 0.68 to 0.72 for discriminating between mild-to-moderate and severe PA as determined in the DBPCFC/anaphylaxis subgroup, and did not differ significantly from the AUCs of individual extract- and allergen-based tests (table S5).

Discussion

The current study provides insight into the clinical profiles of subjects with mild-to-moderate and severe probable PA, and quantifies the relative importance of information obtained during diagnostic work-up of PA for prediction of severity. Sex, age at onset of PA, symptoms elicited by skin contact with peanut, family atopy, atopic dermatitis (ever), birch pollen allergy, HDM allergy, latex allergy, peanut extract SPT wheal ratio, and IgE levels to peanut extract, Ara h 1, 2/6 and 8, were found to be independently associated with severity, of which only birch pollen allergy and IgE to Ara h 8 were associated with a mild-to-moderate phenotype. A model combining these determinants led to optimal discrimination between mild-to-moderate and severe probable PA (cross-validated AUC 0.75), but measures of peanut sensitisation contributed only limited predictive value in addition to clinical background determinants alone.

It was intriguing that some of the strongest independent predictors from clinical background associated with severe probable PA were skin-related: having symptoms elicited by skin contact with peanut, atopic dermatitis (ever), or latex allergy (Figure 1). Exposure to food allergens in early life via the skin has been proposed to play an important role in allergic sensitisation.[31] Loss-of-function mutations in genes encoding the skin component filaggrin are related to a disrupted skin barrier, are often seen in children with atopic dermatitis, and are associated with IgE sensitisation and allergy to foods in general,[32, 33] and peanut specifically.[33-36] Little has been reported on the relationship between atopic dermatitis and severity of food allergic reactions, but in agreement with our findings, Van der Leek *et al*. also found that peanut allergic children reporting skin contact reactions to peanut were more likely to experience severe peanut allergic reactions.[37] Our previous prediction models developed for hazelnut and walnut allergy also contained atopic dermatitis (hazelnut and walnut), latex allergy (hazelnut), and symptoms elicited by skin contact (walnut) as predictors for severe

reactivity.[20, 21] Altogether, cutaneous sensitivity may be a marker for severe food allergy.

The only independent determinants to be associated with mild-to-moderate probable PA, were birch pollen allergy and sensitisation to Ara h 8, a PR-10 protein homologous to major birch pollen allergen Bet v 1. Birch pollen-related FA is one of the most common types of plant source FA in adults in (especially Northern and Central) Europe and generally presents with mild (often isolated oral allergy) symptoms.[1, 38] The frequent occurrence of this condition is reflected in our study population - 41% of subjects with mild-to-moderate PA had isolated OAS, of which 73% were sensitised to Ara h 8, making birch pollen-related PA plausible.

Interestingly, all subjects with probable PA who were not sensitised to peanut extract in SPT or ImmunoCAP, were found to be sensitised to Ara h 8 (Table S2). The peanut PR-10 protein is apparently underrepresented in peanut extract. This suggests that subjects with birch pollen-related PA are not well detected with peanut extract, which partly explains why SPT wheal size and IgE level to peanut extract are associated with severe probable PA. Our findings were similar for walnut allergy, where the majority of subjects with negative extract-based tests were sensitised to walnut PR-10 protein Jug r 5.[21] In contrast, sensitisation to hazelnut extract, which is spiked with hazelnut PR-10 protein Cor a 1, is more common in subjects with mild-to-moderate hazelnut allergy.[20] In the awareness that the association between extract-based testing and severity of PA was limited, these observations still underline the importance of understanding the allergen composition of food extracts for clinical interpretation of extract-based test results.

Our data showed that levels of IgE to peanut storage proteins Ara h 1, 2/6 and 3 (and also to other legumes', tree nuts' and seeds' storage proteins) were significantly higher in subjects with severe probable PA, in accordance with several previous studies in primarily adult populations.[7, 16, 39, 40] Of the individual tests for IgE sensitisation to peanut extract or components, IgE to Ara h 2/6 had the strongest ability to discriminate between mild-to-moderate and severe probable PA, but the AUC only reached 0.64 (table S5). This observation indicated that, although IgE levels to Ara h 1, 2/6 and 3 correlated significantly with severity, they could not be used independently to predict severity of probable PA in an individual patient. These findings were in support of those previously reported by Klemans *et al*, who also found that IgE to Ara h 2 was associated with severity of PA in their adult population, but could not discriminate well between mild and severe PA in individual patients, with comparable AUCs of 0.58 for severity based on patient history and 0.65 for severity based on DBPCFC.[7]

In the current study, IgE to peanut extract (in both SPT and ImmunoCAP) and to peanut storage proteins Ara h 1 and Ara h2/6, were found to contribute to an increased risk of severe probable PA in multivariable analyses. However, the negligible increase of the AUC after addition of measures of peanut IgE sensitisation (in model II and III) to information from clinical background (model I), implies that clinical background is most useful for predicting severity of probable PA in an individual patient, and patient history can detect most of the variation explained by differences in IgE levels. To our knowledge, only one previous study, by Petterson *et al.* assessed prediction of *severity* of PA using a combination of variables from clinical background and measures of IgE sensitisation (only peanut extract), but in a paediatric population and using linear regression.[22] They conclude that reaction severity is largely unpredictable, but the differences in methodological approach prevent in-depth comparison to our study results. Some studies suggest that other laboratory predictors than taken into account in our study may also contribute to prediction of severe PA, such as epitope diversity (combined rather than isolated recognition of Ara h 1, 2 and 3),[41, 42] sIgE/sIgG$_4$ ratios,[15, 43] or results from the basophil activation test (BAT).[15, 44] Especially the BAT has recently been explored independently and as part of multivariable approaches for prediction of PA severity in several studies. The promising results, albeit in primarily paediatric populations, suggest that the BAT has the potential to truly enhance prediction of PA severity in the coming years.[43, 45-48]

Other recommendations for improving prediction of severity of PA in future research, building on the findings in the current study, would be to use ImmunoCAP rather than the less sensitive microarray for measurement of component-specific IgE, and to include other potentially relevant peanut components, like profilin Ara h 5, 2S albumin Ara h 7 and lipid transfer protein Ara h 9.[16, 19, 49, 50] The latter is a major peanut allergen in Southern Europe and may contribute to higher predictive accuracy in those regions.[16, 51] The results from the current studies are, for the largest part, based on subjects from birch-endemic areas. It is important to realise that we made the conscious decision to include subjects with likely birch pollen-related PA in our population, even though pollen-related food allergy is considered a separate clinical entity by some. Exclusion of these patients would make the clinical relevance of our findings much more limited for the average presenting outpatient population in most countries in this study. In future research, further specification of the study population to only include subjects from regions with similar pollen exposure, or only children or adults, could further refine prediction and clinical applicability of findings.

One might consider the main limitation of our study to be that the primary outcome measure was based on self-reported symptoms rather than symptoms during challenge testing. For this reason, we made sure only subjects with IgE

sensitisation to peanut extract or components were included, and additionally explored the results of our analyses in the subgroup of subjects who underwent challenge testing or were excluded from challenge testing because of a history of severe anaphylaxis. We found it reassuring that there was considerable overlap in independent predictors. It was surprising that Ara h 8 tended to be associated with a more severe phenotype of PA in the DBPCFC/anaphylaxis group, for which we have no clear explanation other than that the subgroup may not accurately represent an unselected population of subjects with PA. We also point out that reaction severity based on self-reported symptoms may better reflect real life than reaction severity estimated by challenge, because of challenge exclusion and stopping criteria, and the disinclination of patients who experience severe reactions to undergo or complete a burdensome challenge. As a result of the latter, dietary avoidance advice and medical prescriptions in daily practice are often decided based on clinical history and measurements of IgE sensitisation, making models predicting severity of probable PA particularly interesting. We used penalised regression to prevent overfitting of our models to the population in which they were developed, but as with all prediction models, the models should still be validated in an external population.

To our knowledge, this is the first study to evaluate the individual and combined contribution of clinical background, extract-based tests, and CRD, for prediction of PA severity in a primarily adult population. The penalised regression method increases the generalisability of results, and the standardised approach facilitates comparison to similar models designed for tree nuts. Although not superimposable, clinical profiles for hazelnut and walnut displayed clear similarities. However, it was interesting to observe that measurements of IgE sensitisation contributed minimally to prediction of severity of probable PA, in contrast to the models for severity of hazelnut or walnut allergy. Clinical background determinants were clearly most valuable for predicting severity of probable PA in an individual patient. It will be interesting to validate and further expand these models in other populations to increase predictive accuracy, and to develop models according to the same approach in other food groups for comparative purposes.

Acknowledgements

We would like to thank all the patients for their participation in the study, and ALK Abello (Madrid, Spain) for their generous gift of SPT reagents. We acknowledge the support by the 6th and 7th Framework Programmes of the EU, for EuroPrevall (FP6-FOOD-CT-2005-514000) and iFAAM (Grant agreement no. 312147), respectively.

References

1. Turner PJ, Baumert JL, Beyer K, Boyle RJ, Chan CH, Clark AT, et al. Can we identify patients at risk of life-threatening allergic reactions to food? Allergy. 2016;71(9):1241-55.
2. Greenhawt M, Shaker M, Wang J, Oppenheimer JJ, Sicherer S, Keet C, et al. Peanut allergy diagnosis: A 2020 practice parameter update, systematic review, and GRADE analysis. J Allergy Clin Immunol. 2020.
3. Wainstein BK, Studdert J, Ziegler M, Ziegler JB. Prediction of anaphylaxis during peanut food challenge: usefulness of the peanut skin prick test (SPT) and specific IgE level. Pediatr Allergy Immunol. 2010;21(4 Pt 1):603-11.
4. Summers CW, Pumphrey RS, Woods CN, McDowell G, Pemberton PW, Arkwright PD. Factors predicting anaphylaxis to peanuts and tree nuts in patients referred to a specialist center. J Allergy Clin Immunol. 2008;121(3):632-8 e2.
5. Ta V, Weldon B, Yu G, Humblet O, Neale-May S, Nadeau K. Use of Specific IgE and Skin Prick Test to Determine Clinical Reaction Severity. Br J Med Med Res. 2011;1(4):410-29.
6. Klemans RJ, Otte D, Knol M, Knol EF, Meijer Y, Gmelig-Meyling FH, et al. The diagnostic value of specific IgE to Ara h 2 to predict peanut allergy in children is comparable to a validated and updated diagnostic prediction model. J Allergy Clin Immunol. 2013;131(1):157-63.
7. Klemans RJ, Broekman HC, Knol EF, Bruijnzeel-Koomen CA, Otten HG, Pasmans SG, et al. Ara h 2 is the best predictor for peanut allergy in adults. J Allergy Clin Immunol Pract. 2013;1(6):632-8 e1.
8. Nicolaou N, Poorafshar M, Murray C, Simpson A, Winell H, Kerry G, et al. Allergy or tolerance in children sensitised to peanut: prevalence and differentiation using component-resolved diagnostics. J Allergy Clin Immunol. 2010;125(1):191-7 e1-13.
9. Eller E, Bindslev-Jensen C. Clinical value of component-resolved diagnostics in peanut-allergic patients. Allergy. 2013;68(2):190-4.
10. Beyer K, Grabenhenrich L, Hartl M, Beder A, Kalb B, Ziegert M, et al. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. Allergy. 2015;70(1):90-8.
11. Martinet J, Couderc L, Renosi F, Bobee V, Marguet C, Boyer O. Diagnostic Value of Antigen-Specific Immunoglobulin E Immunoassays against Ara h 2 and Ara h 8 Peanut Components in Child Food Allergy. Int Arch Allergy Immunol. 2016;169(4):216-22.
12. van Veen LN, Heron M, Batstra M, van Haard PMM, de Groot H. The diagnostic value of component-resolved diagnostics in peanut allergy in children attending a Regional Paediatric Allergology Clinic. BMC Pediatr. 2016;16:74.
13. van Erp FC, Knol EF, Pontoppidan B, Meijer Y, van der Ent CK, Knulst AC. The IgE and basophil responses to Ara h 2 and Ara h 6 are good predictors of peanut allergy in children. J Allergy Clin Immunol. 2017;139(1):358-60 e8.
14. Datema MR, Eller E, Zwinderman AH, Poulsen LK, Versteeg SA, van Ree R, et al. Ratios of specific IgG4 over IgE antibodies do not improve prediction of peanut allergy nor of its severity compared to specific IgE alone. Clin Exp Allergy. 2019;49(2):216-26.
15. Song Y, Wang J, Leung N, Wang LX, Lisann L, Sicherer SH, et al. Correlations between basophil activation, allergen-specific IgE with outcome and severity of oral food challenges. Ann Allergy Asthma Immunol. 2015;114(4):319-26.
16. Ballmer-Weber BK, Lidholm J, Fernandez-Rivas M, Seneviratne S, Hanschmann KM, Vogel L, et al. IgE recognition patterns in peanut allergy are age dependent: perspectives of the EuroPrevall study. Allergy. 2015;70(4):391-407.
17. Blumchen K, Beder A, Beschorner J, Ahrens F, Gruebl A, Hamelmann E, et al. Modified oral food challenge used with sensitisation biomarkers provides more real-life clinical thresholds for peanut allergy. J Allergy Clin Immunol. 2014;134(2):390-8.
18. Deschildre A, Elegbede CF, Just J, Bruyere O, Van der Brempt X, Papadopoulos A, et al. Peanut-allergic patients in the MIRABEL survey: characteristics, allergists' dietary advice and lessons from real life. Clin Exp Allergy. 2016;46(4):610-20.

19. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy. 2014;69(8):1008-25.
20. Datema MR, van Ree R, Asero R, Barreales L, Belohlavkova S, de Blay F, et al. Component-resolved diagnosis and beyond: Multivariable regression models to predict severity of hazelnut allergy. Allergy. 2018;73(3):549-59.
21. Lyons SA, Datema MR, Le TM, Asero R, Barreales L, Belohlavkova S, et al. Walnut Allergy Across Europe: Distribution of Allergen Sensitisation Patterns and Prediction of Severity. J Allergy Clin Immunol Pract. 2020.
22. Pettersson ME, Koppelman GH, Flokstra-de Blok BMJ, Kollen BJ, Dubois AEJ. Prediction of the severity of allergic reactions to foods. Allergy. 2018;73(7):1532-40.
23. DunnGalvin A, Daly D, Cullinane C, Stenke E, Keeton D, Erlewyn-Lajeunesse M, et al. Highly accurate prediction of food challenge outcome using routinely available clinical data. J Allergy Clin Immunol. 2011;127(3):633-9 e1-3.
24. Fernandez-Rivas M, Barreales L, Mackie AR, Fritsche P, Vazquez-Cortes S, Jedrzejczak-Czechowicz M, et al. The EuroPrevall outpatient clinic study on food allergy: background and methodology. Allergy. 2015;70(5):576-84.
25. Hoffmann-Sommergruber K, Mills EN, Vieths S. Coordinated and standardized production, purification and characterization of natural and recombinant food allergens to establish a food allergen library. Mol Nutr Food Res. 2008;52 Suppl 2:S159-65.
26. Cochrane SA, Salt LJ, Wantling E, Rogers A, Coutts J, Ballmer-Weber BK, et al. Development of a standardized low-dose double-blind placebo-controlled challenge vehicle for the EuroPrevall project. Allergy. 2012;67(1):107-13.
27. Muraro A, Fernandez-Rivas M, Beyer K, Cardona V, Clark A, Eller E, et al. The urgent need for a harmonized severity scoring system for acute allergic reactions. Allergy. 2018;73(9):1792-800.
28. Sampson HA. Anaphylaxis and emergency treatment. Pediatrics. 2003;111(6 Pt 3):1601-8.
29. Tibshirani R. Regression Shrinkage and Selection via the Lasso. J Roy Stat Soc B. 1996;58(1):267-88.
30. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988;44(3):837-45.
31. Lack G. Update on risk factors for food allergy. J Allergy Clin Immunol. 2012;129(5):1187-97.
32. Tsakok T, Marrs T, Mohsin M, Baron S, du Toit G, Till S, et al. Does atopic dermatitis cause food allergy? A systematic review. J Allergy Clin Immunol. 2016;137(4):1071-8.
33. Venkataraman D, Soto-Ramirez N, Kurukulaaratchy RJ, Holloway JW, Karmaus W, Ewart SL, et al. Filaggrin loss-of-function mutations are associated with food allergy in childhood and adolescence. J Allergy Clin Immunol. 2014;134(4):876-82 e4.
34. Hill DJ, Sporik R, Thorburn J, Hosking CS. The association of atopic dermatitis in infancy with immunoglobulin E food sensitisation. J Pediatr. 2000;137(4):475-9.
35. Sampson HA, Broadbent KR, Bernhisel-Broadbent J. Spontaneous release of histamine from basophils and histamine-releasing factor in patients with atopic dermatitis and food hypersensitivity. N Engl J Med. 1989;321(4):228-32.
36. Brown SJ, Asai Y, Cordell HJ, Campbell LE, Zhao Y, Liao H, et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. J Allergy Clin Immunol. 2011;127(3):661-7.
37. Vander Leek TK, Liu AH, Stefanski K, Blacker B, Bock SA. The natural history of peanut allergy in young children and its association with serum peanut-specific IgE. J Pediatr. 2000;137(6):749-55.
38. Werfel T, Asero R, Ballmer-Weber BK, Beyer K, Enrique E, Knulst AC, et al. Position paper of the EAACI: food allergy due to immunological cross-reactions with common inhalant allergens. Allergy. 2015;70(9):1079-90.
39. Peeters KA, Koppelman SJ, van Hoffen E, van der Tas CW, den Hartog Jager CF, Penninks AH, et al. Does skin prick test reactivity to purified allergens correlate with clinical severity of peanut allergy? Clin Exp Allergy. 2007;37(1):108-15.

40. Moverare R, Ahlstedt S, Bengtsson U, Borres MP, van Hage M, Poorafshar M, et al. Evaluation of IgE antibodies to recombinant peanut allergens in patients with reported reactions to peanut. Int Arch Allergy Immunol. 2011;156(3):282-90.
41. Flinterman AE, Knol EF, Lencer DA, Bardina L, den Hartog Jager CF, Lin J, et al. Peanut epitopes for IgE and IgG4 in peanut-sensitised children in relation to severity of peanut allergy. J Allergy Clin Immunol. 2008;121(3):737-43 e10.
42. Shreffler WG, Beyer K, Chu TH, Burks AW, Sampson HA. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. J Allergy Clin Immunol. 2004;113(4):776-82.
43. Santos AF, Du Toit G, O'Rourke C, Becares N, Couto-Francisco N, Radulovic S, et al. Biomarkers of severity and threshold of allergic reactions during oral peanut challenges. J Allergy Clin Immunol. 2020;146(2):344-55.
44. Santos AF, Du Toit G, Douiri A, Radulovic S, Stephens A, Turcanu V, et al. Distinct parameters of the basophil activation test reflect the severity and threshold of allergic reactions to peanut. J Allergy Clin Immunol. 2015;135(1):179-86.
45. Chinthrajah RS, Purington N, Andorf S, Rosa JS, Mukai K, Hamilton R, et al. Development of a tool predicting severity of allergic reaction during peanut challenge. Ann Allergy Asthma Immunol. 2018;121(1):69-76 e2.
46. Cottel N, Saf S, Bourgoin-Heck M, Lambert N, Amat F, Poncet P, et al. Two Different Composite Markers Predict Severity and Threshold Dose in Peanut Allergy. J Allergy Clin Immunol Pract. 2020.
47. Buyuktiryaki B, Santos AF. Food allergy severity predictions based on cellular in vitro tests. Expert Rev Mol Diagn. 2020.
48. Santos AF. Food allergy severity prediction: quite a way to go yet? Expert Rev Clin Immunol. 2020;16(6):543-6.
49. Palladino C, Breiteneder H. Peanut allergens. Mol Immunol. 2018;100:58-70.
50. Blankestijn MA, Otten HG, Suer W, Weimann A, Knol EF, Knulst AC. Specific IgE to peanut 2S albumin Ara h 7 has a discriminative ability comparable to Ara h 2 and 6. Clin Exp Allergy. 2018;48(1):60-5.
51. Vereda A, van Hage M, Ahlstedt S, Ibanez MD, Cuesta-Herranz J, van Odijk J, et al. Peanut allergy: Clinical and immunologic differences among patients from 3 different geographic regions. J Allergy Clin Immunol. 2011;127(3):603-7.

Supplemental files

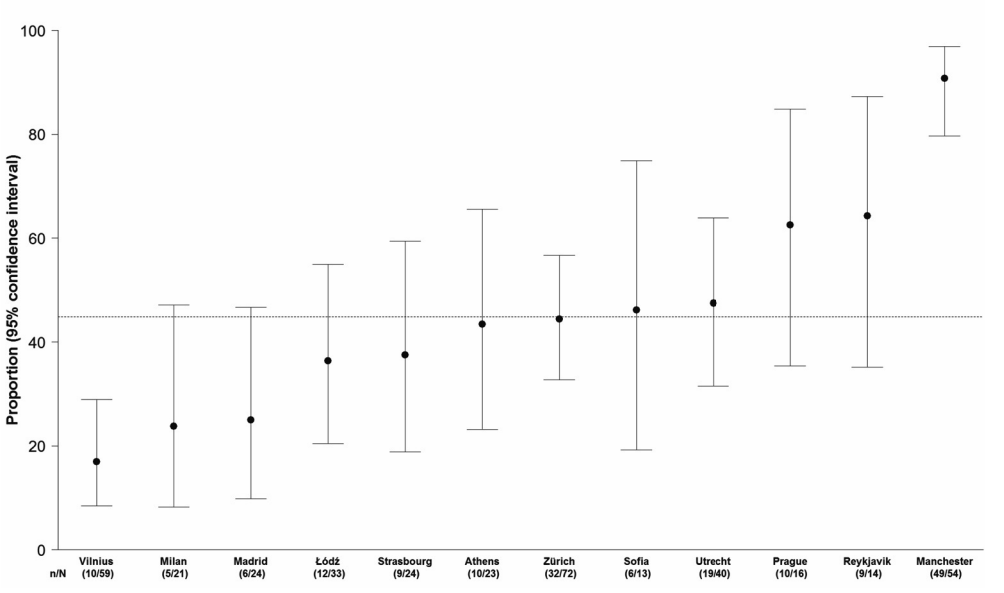

Figure S1. Occurrence of severe probable PA in European cities

Table S1. Missing data in variables included for Lasso regression

	Number of missings
Age at visit	0
Sex	0
Age at onset of symptoms peanut allergy	8
Symptoms upon skin contact peanut	55
Family history of atopic disease	7
Atopic dermatitis (ever)	6
Birch pollen allergy (reported)	8
IgE birch extract	20
SPT birch extract	22
Grass pollen allergy (reported)	8
IgE grass extract	20
SPT grass extract	22
Mugwort pollen allergy (reported)	8
IgE mugwort extract	20
SPT mugwort extract	25
House dust mite allergy (reported)	32
IgE house dust mite extract	20
SPT house dust mite extract	21
Latex allergy (reported)	33
IgE latex	42
IgE cat	20
IgE dog	42
SPT peanut extract	6
IgE peanut extract (ImmunoCAP)	17
Ara h 1 (microarray)	73
Ara h 2/6 (microarray)	73
Ara h 3/3.02 (microarray)	73
Ara h 8 (microarray)	73

Total N = 393. Values for these missing data were estimated using multiple imputation procedures, for which all of the above determinants were included as covariates, along with reported symptoms (0 missings), centre, and reported allergy, SPT, ImmunoCAP and microarray results for foods other than peanut. *SPT, skin prick test.*

Table S2. IgE to peanut components in subjects with negative peanut SPT and ImmunoCAP

	Negative SPT and ImmunoCAP peanut extract (N=27)	
	N microarray positive*	**IgE level**, *median (IQR)*
Ara h 1	2/27	0.30; 0.31
Ara h 2/6	0/27	NA
Ara h 3	0/27	NA
Ara h 8	27/27	0.51 (0.68-4.10)

** IgE ≥0.3 ISU/L. NA, not applicable*, because 0 subjects sensitised.

Table S3. Sensitisation to food extracts other than peanut in subjects with mild-to-moderate and severe probable PA

Food extract	Measurement	Mild-to-moderate (N=216)		Severe (N=177)		p
Soybean	n positive/N total, %	94/209	45.0	83/167	49.7	0.419
	IgE level median, IQR	0.25	0.05-0.79	0.35	0.07-1.54	**0.022**
Lentil	n positive/N total, %	89/208	42.8	83/168	49.4	0.240
	IgE level median, IQR	0.24	0.05-0.90	0.33	0.06-1.95	**0.020**
Hazelnut	n positive/N total, %	168/208	80.8	135/168	80.4	1.000
	IgE level median, IQR	4.74	0.73-24.89	3.55	0.64-19.53	0.065
Walnut	n positive/N total, %	91/208	43.8	76/168	45.2	0.854
	IgE level median, IQR	0.25	0.04-1.36	0.27	0.06-1.09	0.512
Sesame seed	n positive/N total, %	111/208	53.4	106/167	63.5	0.062
	IgE level median, IQR	0.40	0.15-1.40	0.71	0.21-2.08	0.461
Peach	n positive/N total, %	168/207	81.2	107/168	63.7	**<0.001***
	IgE level median, IQR	2.59	0.59-7.20	1.24	0.18-4.35	**0.006**
Apple	n positive/N total, %	147/207	71.0	104/168	61.9	0.079
	IgE level median, IQR	1.39	0.27-4.97	0.77	0.16-2.86	**0.038**
Kiwi	n positive/N total, %	109/207	52.7	95/168	56.5	0.517
	IgE level median, IQR	0.40	0.10-1.68	0.42	0.08-1.27	0.074
Tomato	n positive/N total, %	111/208	53.4	87/168	51.8	0.841
	IgE level median, IQR	0.41	0.10-1.91	0.38	0.09-1.52	0.128
Carrot	n positive/N total, %	121/208	58.2	95/168	56.5	0.832
	IgE level median, IQR	0.51	0.11-2.54	0.55	0.08-1.76	0.064
Celery	n positive/N total, %	122/208	58.7	97/168	57.7	0.941
	IgE level median, IQR	0.68	0.14-2.73	0.54	0.08-1.98	**0.043**

Sensitisation was considered positive at IgE levels ≥0.35 kU/L. The p-value indicates the difference between mild-to-moderate and severe probable peanut allergy subjects. **Bold** indicates $p<0.05$. *Differences remained significant after Bonferroni correction.

Table S4. Sensitisation to food allergens other than peanut allergens in subjects with mild-to-moderate and severe probable PA

Food source	Allergen	Mild-to-moderate (N=216)		Severe (N=177)		p
		n positive/N total	%	n positive/N total	%	
Soybean	Gly m 4	95/176	54.0	45/144	31.2	**<0.001***
	Gly m 5	7/176	4.0	24/144	16.7	**<0.001***
	Gly m 6	6/176	3.4	25/144	17.4	**<0.001***
Hazelnut	Cor a 1.0401	115/176	65.3	71/144	49.3	**0.005**
	Cor a 2	32/176	18.2	17/144	11.8	0.156
	Cor a 8	26/176	14.8	23/144	16.0	0.888
	Cor a 9	30/176	17.0	23/144	16.0	0.916
	Cor a 11	2/176	1.1	17/144	11.8	**<0.001***
Walnut	Jug r 2	10/176	5.7	20/144	13.9	**0.021**
	Jug r 4	2/176	1.1	7/144	4.9	0.084
Sesame seed	Ses i 1	0/176	0.0	5/144	3.5	**0.018**
	Ses i 2	1/176	0.005	5/144	3.5	0.094
	Ses i 3	35/176	19.9	24/144	16.7	0.553
Peach	Pru p 1	108/176	61.4	74/144	51.4	0.093
	Pru p 3	34/176	19.3	16/144	11.1	0.063
Apple	Mal d 1	82/176	46.6	49/144	34.0	**0.031**
	Mald 2	2/176	1.1	2/144	1.4	1.000
	Mal d 3	30/176	17.0	21/144	14.6	0.656
	Mal d 4	34/176	19.3	29/144	20.1	0.966
Kiwi	Act d 1	4/176	2.3	10/144	6.9	0.054
Tomato	Lyc e 3	22/176	12.5	15/144	10.4	0.686
Carrot	Dau c 1.0201	24/176	13.6	24/144	16.7	0.550
	Dau c 1.0103	9/176	5.1	8/144	5.6	1.000
	Dauc 4	38/176	21.6	32/144	22.2	1.000
Celery	Api g 1.01	36/176	20.5	24/144	16.7	0.472
	Api g 4	46/176	26.1	39/144	27.1	0.949
	Api g 5	37/176	21.0	26/144	18.1	0.601

Sensitisation was considered positive at IgE levels ≥0.3 ISU/L. The p-value indicates the difference between mild-to-moderate and severe probable PA subjects. **Bold** indicates p<0.05. *Differences remained significant after Bonferroni correction.

Table S5. Area under the ROC-curve of individual and combined tests for prediction of severity of PA

Test	Probable peanut allergy		DBPCFC/anaphylaxis	
	AUC	**95%-CI**	**AUC**	**95%-CI**
Peanut extract				
SPT	0.63	0.61-0.65	0.63	0.60-0.67
ImmunoCAP	0.63	0.62-0.65	0.72	0.69-0.75
Peanut allergens (microarray)				
Ara h 1	0.62	0.59-0.64	0.70	0.66-0.75
Ara h 2/6	0.64	0.61-0.66	0.70	0.60-0.81
Ara h 3/3.02	0.60	0.58-0.63	0.69	0.64-0.73
Ara h 8	0.54	0.50-0.61	0.47	0.43-0.51
CRD only*				
Ara h 2/6 & Ara h 8*	0.65	0.63-0.66	-	-
Ara h 1 & Ara h 2/6*	-	-	0.70	0.66-0.75
Models**				
Model I	0.74[†]	0.72-0.75[†]	0.68	0.65-0.72
Model II	0.74[†]	0.73-0.76[†]	0.72	0.68-0.75
Model III	0.75[†]	0.74-0.77[†]	0.71	0.67-0.74

The areas under the curve (AUC) and the 95% confidence intervals (95%-CI) indicate the ability to discriminate between patients with mild-to-moderate and patients with severe allergic symptoms to peanuts. AUCs for SPT, peanut extract and allergen components by microarray were averaged over the 10 imputed datasets. *****Allergens selected by Lasso regression when combining peanut allergens measured by microarray. For probable peanut allergy, the model included Ara h 2/6 and Ara h 8. For the DBPCFC group, the model included Ara h 1 and Ara h 2/6. ******As shown in table 3. [†]Significantly larger ($p<0.001$) than the AUC of individual extract-based and allergen-based tests (De Long's test). *CI, confidence interval; DBPCFC, double-blind placebo-controlled food challenge; SPT, skin prick test.*

Table S6. Accuracy of individual diagnostic tests and models for severity of peanut allergy

Individual test	Positivity threshold		Mild-to-moderate	Severe	Sensitivity	95%-CI	Specificity	95%-CI	PPV	95%-CI	NPV	95%-CI
SPT peanut extract	**0.50**	≥	173	143	81.7	75.2-87.1	18.4	13.4-24.3	45.2	39.7-50.9	54.9	42.7-66.8
		<	39	32								
	0.29	≥	181	150	**85.1**	**79.6-90.5**	14.6	10.2-20.1	45.3	39.8-50.8	55.4	41.5-68.7
		<	31	25								
	2	≤	11	34	19.4	13.8-26.1	**94.8**	**90.9-97.4**	75.6	60.5-87.1	58.8	53.4-64.0
		>	201	141								
ImmunoCAP peanut extract	**0.35**	≥	144	140	83.8	77.4-89.1	31.1	24.9-37.9	49.3	43.3-55.3	70.7	60.2-79.7
		<	65	27								
	0.007	≥	182	158	**94.6**	**90.0-97.5**	12.9	8.7-18.2	46.5	41.1-51.9	75	57.8-87.9
		<	27	9								
	25.7	≥	10	33	19.8	14.0-26.6	**95.2**	**91.4-97.7**	76.7	61.4-88.2	59.8	54.3-65.1
		<	199	134								
Microarray Ara h 1	**0.30**	≥	26	54	37.5	29.6-46.0	87.5	81.7-92.0	71.0	59.5-80.9	63.1	56.7-69.2
		<	150	90								
	0.10	≥	34	63	**43.8**	**35.5-52.3**	80.7	74.1-86.2	65.0	54.6-74.4	63.7	5.0-70.0
		<	142	81								
	0.85	≥	8	36	25.0	18.2-32.9	**95.5**	**91.3-98.0**	81.8	67.3-91.8	**60.9**	**54.8-66.7**
		<	168	108								
Microarray Ara h 2/6	**0.30**	≥	19	56	38.9	30.9-47.4	89.2	83.7-93.4	74.7	63.3-84.0	64.1	57.7-70.1
		<	157	88								
	0.11	≥	19	59	**41.0**	**32.9-49.8**	89.2	83.7-93.4	75.6	64.6-84.7	64.9	58.5-70.9
		<	157	85								
	8.27	≥	8	34	23.6	16.9-31.4	**95.5**	**91.2-98.0**	81.0	65.9-91.4	60.4	54.4-66.2
		<	168	110								
Microarray Ara h 3	**0.30**	≥	10	43	29.9	22.5-38.0	94.3	89.8-97.2	81.1	68.0-90.6	62.2	56.1-68.0
		<	166	101								
	0.19	≥	12	47	**32.6**	**25.1-40.9**	93.2	88.4-96.4	79.7	67.2-89.0	62.8	56.7-68.7
		<	164	97								
	0.26	≥	8	33	22.9	16.3-30.6	**95.5**	**91.2-98.0**	80.5	65.1-91.2	60.2	54.2-66.0
		<	168	111								
Microarray Ara h 8	**0.30**	≥	112	67	46.5	38.2-55.0	36.4	29.3-43.9	37.4	30.3-45.0	45.4	37.0-54.0
		<	64	77								
	0.10	≥	119	86	**59.7**	**51.2-67.8**	32.4	25.5-39.8	42.0	35.1-49.0	49.6	40.1-59.0
		<	57	58								
	8.53	≥	8	1	0.7	0.3-8.0	**95.5**	**91.2-98.0**	11.1	0.3-48.2	54.0	48.3-59.7
		<	168	143								

Table S6. Accuracy of individual diagnostic tests and models for severity of peanut allergy (continued)

Model	Positivity threshold		Mild-to-moderate	Severe	Sensitivity	95%-CI	Specificity	95%-CI	PPV	95%-CI	NPV	95%-CI
Model I	**0.23**	≥	135	110	**94.8**	**89.1-98.1**	15.1	9.9-21.6	44.9	38.6-51.4	80.0	61.4-92.3
		<	24	6								
	0.61	≥	8	41	35.3	26.7-44.8	**95.0**	**90.3-97.8**	83.7	70.3-92.7	66.8	60.3-72.9
		<	151	75								
Model II	**0.24**	≥	128	104	**95.4**	**89.6-98.5**	16.9	11.3-23.8	44.8	38.3-51.5	83.9	66.3-94.6
		<	26	5								
	0.64	≥	7	41	37.6	28.5-47.4	**95.5**	**90.7-95.2**	85.4	72.3-93.9	68.4	61.7-74.5
		<	147	68								
Model III	**0.25**	≥	132	104	**95.4**	**89.6-98.5**	14.3	9.2-20.8	44.1	37.6-50.7	81.5	61.9-93.7
		<	22	5								
	0.63	≥	7	4	36.7	27.7-46.5	**95.5**	**90.8-98.2**	85.1	71.7-93.8	68.1	61.4-74.2
		<	147	69								

Measures of accuracy were calculated for each of the individual diagnostic tests, and for the models on clinical background variables (model I), clinical background variables + sensitisation to peanut extract in SPT or ImmunoCAP (model II), and clinical background variables + sensitisation to peanut extract + sensitisation to peanut components (model III). The three rows of threshold values given for each diagnostic test respectively indicate the cutoffs generally used in clinical practice, corresponding with a high sensitivity (closest to 95%), and corresponding with a high specificity (closest to 95%). **Bold** indicates the sensitivity and specificity estimates closest to 95%. *CI, confidence interval; CRD, component-resolved diagnostics; NPV, negative predictive value; PPV, positive predictive value; SPT, skin prick test.*

Table S7. Characteristics of subjects who underwent DBPCFC or had severe anaphylaxis to peanut

	No or mild-to-moderate symptoms (N=47)	Severe symptoms (N=44)	p
Demographics			
Age in years, *mean (±SD)*	26.0 (±9.9)	20.6 (±9.8)	0.013
Age < 14 years	6/47 (12.8)	11/44 (25.0)	0.180
Female sex	26/47 (55.3)	28/44 (63.6)	0.553
Clinical background			
Age at onset of symptoms < 14 years	25/47 (53.2)	35/44 (79.5)	**0.015**
Symptoms upon skin contact with peanut	7/45 (15.6)	17/30 (56.7)	**<0.001***
Family history of atopic disease	22/47 (46.8)	35/44 (79.5)	**0.002**
Atopic dermatitis	15/47 (31.9)	25/44 (56.8)	0.029
Birch pollen allergy[‡]	16/46 (34.8)	12/43 (27.9)	0.639
Grass pollen allergy[‡]	27/46 (58.7)	26/43 (60.5)	1.000
Mugwort pollen allergy[‡]	2/46 (4.3)	6/43 (14.0)	0.149
House dust mite allergy[‡]	21/43 (48.8)	30/43 (69.8)	0.079
Latex allergy[‡]	3/43 (7.0)	4/42 (9.5)	0.713
Cat/dog sensitisation [‡]	31/47 (66.0)	34/43 (79.1)	0.249
Peanut sensitisation[§]			
SPT peanut extract			
Positive	37/46 (80.4)	36/42 (85.7)	0.708
Allergen/histamine wheal ratio, *median (IQR)*	0.92 (0.58-1.55)	1.28 (0.92-2.13)	0.238
ImmunoCAP peanut extract			
Positive	37/47 (78.7)	39/41 (95.1)	**0.031**
IgE level, *median (IQR)*	1.33 (0.51-6.17)	5.67 (1.54-57.47)	**0.031**
Microarray peanut allergens			
Ara h 1			
Positive	11/39 (28.2)	24/40 (60.0)	**0.009**
IgE level, *median (IQR)*	0.00 (0.00-0.32)	0.60 (0.00-5.6)	0.059
Ara h 2/6			
Positive	10/39 (25.6)	25/40 (62.5)	**0.002**
IgE level, *median (IQR)*	0.00 (0.00-0.24)	6.28 (0.00-19.34)	**0.014**
Ara h 3/3.02			
Positive	6/39 (15.4)	22/40 (55.0)	**<0.001***
IgE level, *median (IQR)*	0.00 (0.00-0.00)	0.44 (0.00-2.68)	0.088
Ara h 8			
Positive	18/39 (46.2)	10/40 (25.0)	0.084
IgE level, *median (IQR)*	0.00 (0.00-0.40)	0.00 (0.00-0.29)	0.243

Subjects with severe symptoms during DBPCFC (N=1) or life-threatening anaphylaxis based on patient history (N=43) were classified as severe. All measurements are in n/N (%) unless otherwise specified. P-values indicate difference between patients with no or mild-to-moderate and patients with severe symptoms to peanut. **Bold** indicates p<0.05. *Differences remained significant after Bonferroni correction. [‡]Reported symptoms + matching sensitisation by SPT or ImmunoCAP. [§]Not all patients had complete testing for peanut sensitisation. *IQR, interquartile range; SPT, skin prick test.*

Chapter 9

Dietary interventions in pollen-related food allergy: a systematic review

Nutrients. 2018; 10(10):1520

Sarah A. Lyons,* Anne M. van Dijk,* André C. Knulst, Eleonora Alquati, Thuy-My Le, Harmieke van Os-Medendorp

*Contributed equally

Abstract

In practice, it remains unclear what the best dietary approach is in subjects with pollen-related food allergy (PRFA). Our objective was to evaluate the effect of (1) dietary avoidance advice, (2) oral immunotherapy (OIT), (3) (heat) processing, and (4) consumption of hypoallergenic cultivars on frequency, severity, and eliciting dose of pollen-related food allergic reactions. A systematic search was conducted in PubMed, Embase, and Cochrane. All studies performing an *in vivo* investigation of one of the four interventions in adults with PRFA were included. Each study was assessed for quality and validity. Available data on frequency, severity, and eliciting dose of allergic reactions were extracted. Ten studies matched the eligibility criteria. No studies were retrieved on dietary avoidance advice. Two studies (total N = 92) on apple OIT reported that tolerance was induced in 63% and 81% of subjects. Four studies (total N = 116) focused on heat processing. Heating was found to completely eradicate symptoms in 15–71% of hazelnut allergic and 46% of celery allergic individuals. Four studies (total N = 60) comparing low to high allergenic apple cultivars revealed that Santana (and possibly Elise) apples seemed to cause milder reactions than Golden Delicious. In the awareness that overall level of evidence was low, we conclude that OIT, heat processing, and hypoallergenic cultivars may diminish or completely prevent allergic reactions in some but not all subjects with PRFA.

Introduction

Up to 90% of pollen sensitised individuals are allergic to foods that cross-react with pollen.[1–4] This pollen-related food allergy (PRFA) is generally characterised by the rapid onset of oropharyngeal symptoms after ingestion and spontaneous resolution within 30 minutes. Systemic reactions are possible but rare.[5,6] Birch PRFA is most common in Northern and Central Europe, but food allergy due to cross-reactivity with mugwort, grass, and plant weed is also described.[5,6] Frequently involved foods include *Rosaceae* fruits (e.g., apple, peach, cherry), *Apiaceae* vegetables (e.g., carrot, celery), peanut, tree nuts, and soybean.[5,6] The increasing incidence of pollen allergy will probably lead to a further increase in PRFA.[5-9]

Primary dietary therapy for food allergy consists of the avoidance of triggering foods.[5,6,10,11] However, clinical guidelines on PRFA give no specific advice regarding avoidance of cross-reacting foods or foods within the same family, nor with regard to avoidance of traces.[5,6] As a result, the specifics of avoidance recommendations differ per physician. In a survey of Ma *et al*, 9% of US allergists did not impose any diet restrictions, 53% of allergists advised avoidance of triggering foods, 4% recommended avoiding potential cross-reacting foods, and 38% based their treatment on individual patient presentation.[12] It remains unclear what the effect of these varying treatments is on pollen-related food allergic reactions in practice.

Furthermore, the clinical efficacy of other dietary interventions, such as oral immunotherapy (OIT) with food, heat processing, and consumption of low allergenic cultivars on PRFA is unknown. Whereas current guidelines do not recommend pollen immunotherapy to treat PRFA, no guidance is given regarding OIT with food.[5,6,10] A recent study investigating the effectiveness of sublingual immunotherapy with recombinant Mal d 1 allergen extract in pollen-related apple allergic patients, showed that this type of immunotherapy was a safe and effective approach to reduce symptoms.[13] A more practicable dietary therapy comprising OIT with the culprit food, has been systematically evaluated and found to be effective in treating allergy to milk, egg, wheat and peanut.[14,15] Although peanut allergy can be a PRFA due to cross-reaction between birch pollen and peanut component Ara h 8 primarily,[5,16] the efficacy of OIT was not specifically discussed for such subjects, and the role of oral immunotherapy with food in PRFA is still unclear. Clinical guidelines describe that heat processing the culprit food can reduce PRFA symptoms, because major food allergens cross-reacting with tree pollen are heat labile.[5,6] Skin prick tests (SPT) in subjects with PRFA are less often positive with cooked than with raw culprit foods.[17] The extent of skin test positivity also appears to depend on the amount of allergen content in different cultivars of the culprit food,[5,6] which gives the impression that consumption of low allergenic rather than high allergenic cultivars may be a valuable

dietary intervention. However, the effect of heating or consumption of low allergenic cultivars on the allergic symptoms of subjects with PRFA remains to be evaluated.

Therefore, the aim of this review was to evaluate the effect of specific dietary interventions on frequency, severity, and eliciting dose of food allergic reactions in adults with PRFA. Evaluated dietary interventions consisted of (1) dietary avoidance advice, (2) OIT with food, (3) (heat) processing, and (4) consumption of hypoallergenic cultivars.

Materials and methods

Protocol and registration

This systematic literature review was carried out according to a protocol registered in advance in the international prospective register of systematic reviews (PROSPERO), registration number CRD42018103805, and presented following the recommendations of the PRISMA checklist.[18]

Eligibility criteria, information sources, and search

Relevant synonyms for our domain (adults with PRFA) and determinants (dietary avoidance advice, OIT with food, heat processing, and consumption of hypoallergenic cultivars) were combined to develop an extensive search strategy (See supplement 'Search strategy for PubMed search'), which was entered into PubMed, Embase, and The Cochrane Library on 6 July 2018 using keywords and Medical Subject Headings. A broad search terminology for PRFA was used as well as particular terms for relevant plant-related inhalant allergens and for specific foods reported to cross-react with these inhalant allergens in recent position papers by European allergy working groups.[5,6] With regard to dietary avoidance advice, we aimed to find studies on the efficacy of different types of dietary advice in practice. Three predetermined dietary interventions were additionally incorporated in the search: OIT, (heat) processing, and consumption of hypoallergenic cultivars. No study design, date or language restrictions were imposed.

Study selection

After importation of all identified citations into EndNote and removal of duplicates, title and abstract screening, and subsequent full text screening were performed by two independent authors (EA, AMvD). Selection was based on consensus; any discrepancies were resolved by consultation of other reviewers (SAL, HvOM, TML). In case of full text unavailability, we attempted to contact authors via email. References of selected articles, reviews and meta-analyses were hand searched and checked in the Scopus citation database for additional articles of interest.

All articles in English, Dutch, German, French, Spanish, and Italian were assessed. For inclusion, the study population had to meet three criteria: (1) ≥80% of the participants were 18 years or older; (2) subjects had a convincing history of hay fever or a positive SPT or ImmunoCAP to at least one type of pollen extract; and (3) subjects had a history of allergic reactions to foods known to cross-react with pollen as well as sensitisation (SPT or CAP) or positive challenge test to the food concerned. Studies were further assessed if they investigated at least one of the determinants of interest.

Studies evaluating immunotherapy other than OIT with eliciting food were excluded, as were studies where low allergenic cultivars were not compared to high allergenic cultivars. We also eliminated non-original studies (reviews, editorials, and expert opinions), conference abstracts, case studies, animal studies, post-mortem studies, etiologic, diagnostic, and prognostic studies, *in vitro* studies, and *in vivo* studies where allergy was only evaluated by SPT.

Data collection

Two authors (AMvD, SAL) independently collected and recorded study characteristics on a predefined checklist, comprising the items author, setting, time frame, study design, study population, method of intervention, method of outcome measurement, and reported outcomes. In some studies, only a part of the total study population was evaluated, because outcomes regarding our determinants of interest were only available for a subgroup of subjects.

For OIT, we obtained data regarding the frequency of achieved tolerance and tolerated dose at final follow-up. For processing and consumption of hypoallergenic cultivars, data on the number of subjects with no allergic reactions after intervention, on symptom severity, and on the eliciting dose were extracted. In order to improve comparability of results from individual studies, the proportion of subjects with an allergic reaction, the median VAS score for symptom severity, and the median dose eliciting symptoms were calculated from available data where possible.

Risk of bias assessment

The validity of included studies was assessed for the part of the study population considered relevant for our research question. The Robins-I tool[19] was used to evaluate seven potential sources of bias: bias due to confounding, bias in selection of participants into the study, bias in classification of interventions, bias due to deviations from intended interventions, bias due to missing data, bias in measurement of outcomes, and bias in selection of the reported results. Two authors (AMvD, SAL) performed an independent evaluation and discussed disagreements to reach consensus. Each article received a final risk level of 'low risk', 'moderate risk', 'high risk', 'critical risk', or 'no information'.

Synthesis of results

Because of evident heterogeneity in methodology and reporting between studies, it was considered inappropriate and infeasible to pool results. A qualitative synthesis of available results was therefore performed. No statistical analyses were conducted. The overall level of the evidence per study outcome per intervention of interest was assessed using the GRADE system[20] and categorised as high quality, moderate quality, low quality or very low quality.

Results

Study selection

Our search yielded 6081 unique citations (Figure 1). Screening of title, abstract, full-text, and related citations provided ten articles suited to address our research question, including one article found via reference checking.

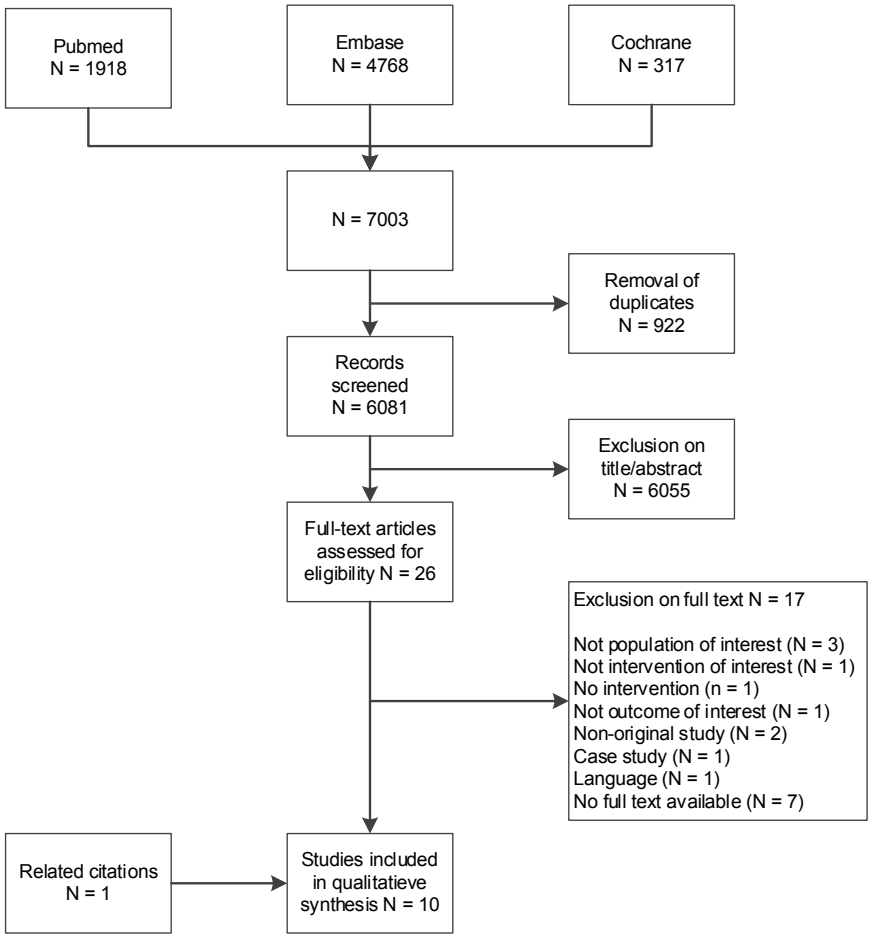

Figure 1. Flowchart

Study characteristics

Details of the ten selected studies can be found in Table 1. They were all conducted in Western Europe and published in French[21] or English[22-30]. All included subjects reported allergy to apple, hazelnut, celery, or carrot and had a history of pollen allergy or were sensitised to birch pollen (and additionally mugwort pollen in one study[23]). No studies were obtained regarding the effect of dietary avoidance advice on frequency, severity, and eliciting dose of allergic reactions.

Two studies, including one randomised controlled trial (RCT), focused on OIT with increasing doses of Golden Delicious apple in a total of 92 subjects.[21,22] Both reported the number of subjects in the intervention group that achieved tolerance to apple and that could consume other *Rosaceae* fruits after a follow-up period of respectively 48 weeks[21] and 8 months[22]. One study provided information on median tolerated dose.[22] Neither study evaluated permanent tolerance, generally referred to as sustained unresponsiveness after a period of discontinuation of regular apple consumption.[14] No studies were found to evaluate OIT with other foods in our study population of interest.

In four studies with 116 subjects in total, authors reported on the effect of heat processing of hazelnut,[25,26] celery,[23,24] carrot[24] and apple[24]. In order to measure the effect, reactions to heated food in double-blind placebo-controlled food challenge (DBPCFC) were compared to reactions to raw food in DBPCFC or history. One study also investigated the effect of processing to celery spice on allergenicity of celery in this patient population.[23] The number of subjects with an allergic reaction to the processed food and their specific symptoms were reported in all studies, along with the information on the tolerated dose in three studies.[23,25,26] Other than heat processing and processing to celery spice, no other methods of processing appeared to have been evaluated *in vivo* by comparative raw *versus* processed food challenge.

Four studies compared the allergenicity of putatively high allergenic to putatively hypoallergenic apple cultivars, primarily assessing the difference in severity of allergic reactions by single- or double-blind food challenge in 60 subjects altogether.[27-30] Golden Delicious (GD), which was classified as the high allergenic cultivar in all studies, was compared to Santana apple in three studies,[28-30] and Elise,[30] Pink Lady,[30] Topaz[29] and G-198/Orim[27] apples in one study each. All studies used various visual analogue scales to assess severity of reactions. Three studies provided information on the proportion of subjects who remained free of symptoms to the various apple cultivars.[27,29,30] The dose eliciting symptoms was discussed in only one study.[28] No studies were found to compare low to high allergenic cultivars for other foods than apple in subjects with PRFA.

Table 1. Study characteristics

Study information	Study design	Relevant study population	Method of intervention	Method of outcome measurement	Outcomes reported
Oral immunotherapy					
Bouvier *et al*; Lyon hospital allergy clinic (F); May 2012 - Feb 2013[21]	NS, comparison of participants' allergic reactions before and after OIT	52 subjects (age 8–63 years; 17% <18 years) with IgE sensitisation to birch pollen and apple; and OAS to *Rosaceae* foods according to history	All subjects underwent OIT with increasing doses of fresh Golden Delicious (GD) apple. 1. Initial dose escalation with 9 doses from 0.1 mg to 16 g. Portion increased every 20–30 min. 2. Build-up phase starting at 16 g and increasing to half an apple (64 g) 3 times per week up until 24 weeks after start. 3. Maintenance phase consisting of half an apple 3 times a week up until 48 weeks after start.	Patient history at 48 weeks follow-up	1. Number of subjects that achieved tolerance of 64 grams of apple after 48 weeks. 2. Number of subjects that was tolerant to other *Rosaceae* fruits after OIT with apple.
Kopac *et al*; University hospital Bern allergy clinic (CH); Dec 2009 – Aug 2010[22]	RCT	40 subjects (age 18–61 years) with IgE sensitisation to birch pollen and Mal d 1; and challenge-confirmed OAS to Golden Delicious apple	27 of 40 subjects underwent OIT with increasing doses of fresh Golden Delicious (GD) apple. 1. Initial dose escalation with doses from 1 g to 128 g. Portion doubled every 5 min. 2. Build-up phase starting at the largest dose tolerated in the preceding phase to whole apple (150–200g). Portion doubled every 2–3 weeks. 3. Maintenance phase commencing when a whole apple was tolerated (average 20 weeks) and consisting of at least three apples per week up until 8 months after the start. 13 of 40 subjects remained untreated and formed the control group.	Patient history at 8 months follow-up	1. Proportion of subjects that achieved tolerance to 128 grams of apple after 8 months. 2. Number of subjects that achieved cross-tolerance to other birch pollen cross-reacting fruits/nuts after OIT with apple.

Table 1. Study characteristics (continued)

Study information	Study design	Relevant study population	Method of intervention	Method of outcome measurement	Outcomes reported
(Heat) processing					
Ballmer-Weber *et al*; University hospital Zurich allergy clinic (CH); Jan 2000 - Feb 2001[23]	NS, comparison of participants' allergic reactions to processed and unprocessed variants of the food	12 subjects (age 21–42 years) with IgE sensitisation to birch pollen (and mugwort pollen in 9/12 subjects) and celery; and allergic reactions to celery according to history	1. Cook celery (110 °C; 15 min) 2. Dehydrate celery (celery spice)	Comparison of DBPCFC with processed celery (12/12 subjects) to DBPCFC with raw celery (10/12 subjects) or convincing history to raw celery (2/12 subjects)	1. Number of subjects with symptoms in response to oral challenge with cooked celery and celery spice 2. Type of symptoms 3. Dose eliciting symptoms
Bohle *et al*; Hannover Medical School Department of Dermatology and Allergology (D); *time NS*[24]	NS, comparison of participants' allergic reactions to processed and unprocessed variants of the food	5 subjects (age 5–37 years; 20% <18 years) IgE sensitised to birch pollen with OAS and worsening of atopic dermatitis to carrot, celery or apple according to history	Celery (1 of 5 subjects): boil until soft Carrot (3 of 5 subjects): boil until soft Apple (1 of 5 subjects): pasteurisation (juice)	Comparison of DBPCFC with processed food (4/5 subjects) or convincing history to processed food (1/5 subjects) to DBPCFC with raw food (5/5 subjects)	1. Number of subjects with OAS in response to oral challenge with cooked carrot or celery or apple 2. Type of symptoms
Hansen *et al*; University hospital Copenhagen (DK), and University hospital Zurich allergy clinics (CH); 1998–2000[25]	NS, comparison of participants' allergic reactions to processed and unprocessed variants of the food	17 subjects (age 14–65 years) with IgE sensitisation to birch pollen and hazelnut; and OAS to hazelnut according to history or challenge	Roast hazelnut (140 °C; 40 min)	Comparison of DBPCFC with roasted hazelnut (17/ 17 subjects) to DBPCFC with raw hazelnut (16/17 subjects) or convincing history to raw hazelnut (1/17 subjects)	1. Number of subjects with symptoms in response to oral challenge with roasted hazelnut 2. Type of symptoms 3. Dose eliciting symptoms
Worm *et al*; University hospital Charité Berlin dermatology outpatient clinic (D); *time NS*[26]	NS, comparison of participants' allergic reactions to processed and unprocessed variants of the food	82 of 132 included subjects (age 21–65 years) with IgE sensitisation to birch pollen and hazelnut; and challenge-confirmed hazelnut allergy	Roast hazelnut (144 °C; time unknown)	Comparison of DBPCFC with roasted hazelnut (20/82 subjects) to DBPCFC with raw hazelnut (82/82 subjects)	1. Number of subjects with symptoms to oral challenge with roasted hazelnut 2. Type of symptoms 3. Dose eliciting symptoms

Table 1. Study characteristics (continued)

Study information	Study design	Relevant study population	Method of intervention	Method of outcome measurement	Outcomes reported
Consumption of hypoallergenic cultivars					
Asero *et al*; *setting NS*; 2004[27]	NS, comparison of participants' allergic reactions to low and high allergenic cultivars	7 of 17 included subjects (age 26–49) with sensitisation to birch pollen and apple; and OAS to apple according to history	Consumption of low allergenic G-198 or Orim apple	Comparison of SBFC with G-198 apple (6/7 subjects) or Orim apple (1/7 subjects) to SBFC with Golden Delicious apple	1. Mean symptom severity score for OAS (Score 0–100) 2. Number of subjects reporting NO symptoms in response to challenge
Bolhaar *et al*; University Medical Centre Utrecht department of dermatology and allergology (NL); *time NS*[28]	NS, comparison of participants' allergic reactions to low and high allergenic cultivars	5 of 23 included subjects (age >18 years) with a history of rhinoconjunctivitis during birch pollen season, sensitisation to apple, and OAS to apple according to history	Consumption of low allergenic Santana apple	Comparison of DBPCFC with Santana apple to DBPCFC with Golden Delicious apple	1. Mean symptom severity score for OAS (VAS 0–100) 2. Quantities needed to provoke similar VAS for Santana apples as for Golden Delicious apples
Kootstra *et al.*; University Medical Centre Groningen allergy outpatient clinic (NL); Feb -May 2005[29]	NS, comparison of participants' allergic reactions to low and high allergenic cultivars	15 subjects (age >18 years) with sensitisation to birch pollen and apple; and challenge-confirmed OAS to apple	Consumption of low allergenic Santana apple	Comparison of SBFC with Santana apple to SBFC with Golden Delicious apple as a positive control and SBFC with Topaz apple as a negative control	1. Maximum symptom severity score (VAS, range not described) at dose 1. 2. Number of subjects reporting NO symptoms in response to oral challenge.
Vlieg-Boerstra *et al*; University Medical Centre Groningen allergy outpatient clinic (NL); 2006 - 2008[30]	NS, comparison of participants' allergic reactions to low and high allergenic cultivars	33 subjects (age 18–52 years) with sensitisation to birch pollen in 32/33 subjects; and challenge-confirmed OAS to apple	Consumption of low allergenic Elise, Santana and Pink Lady apples	Comparison of SBFC with Elise, Santana, Pink Lady and Golden Delicious apple	1. Cumulative symptom severity score (VAS, range not described) at dose 1. 2. Number of subjects reporting NO symptoms in response to oral challenge.

F, France; CH, Switzerland; D, Germany; DK, Denmark; NL, the Netherlands; NS, not specified; OIT, oral immunotherapy; RCT, randomised controlled trial; OAS, oral allergy syndrome; DBPCFC, double-blind placebo-controlled food challenge; SBFC, single-blind food challenge; VAS, visual analogue scale.

Risk of bias assessment

The risk of bias in relation to our study question was moderate to high for all included studies, mainly due to possible confounding, selection bias (generally because only a subgroup of the total study population in some studies was relevant for this review), and bias in outcome measurement. Details of the assessment are presented in Table 2.

Table 2. Risk of bias assessment

	Confounding	Selection	Classification of interventions	Deviations from interventions	Missing data	Outcome measurement	Selection of reported results	Overall risk
Bouvier *et al.*[21]	●	●	●	●	●	●	●	●
Kopac *et al.*[22]	●	●	●	●	●	●	●	●
Ballmer-Weber *et al.*[23]	●	●	●	●	●	●	?	●
Bohle *et al.*[24]	●	●	●	●	●	●	●	●
Hansen *et al.*[25]	●	?	●	●	●	●	●	●
Worm *et al.*[26]	●	●	●	●	●	●	●	●
Asero *et al.*[27]	●	●	●	●	●	●	●	●
Bolhaar *et al.*[28]	●	●	●	●	●	?	●	●
Kootstra *et al.*[29]	●	●	●	●	●	●	●	●
Vlieg-Boerstra *et al.*[30]	●	●	●	●	●	●	●	●

The Robins-I tool was used for risk of bias assessment.[19]

● low risk of bias; ● moderate risk of bias; ● high risk of bias; ? unclear risk of bias

Synthesis of results and level of evidence

A summary of our findings is found in Tables 3 to 5.

Oral immunotherapy

After OIT with Golden Delicious apple, tolerance to apple was achieved in 63–81% of subjects (Table 3). In an RCT, Kopac *et al.* found the frequency of achieved tolerance after 8 months to be significantly higher in the intervention than in the control group (63% *versus* 0%, p = 0.0001). In this study, authors also showed that the median tolerated dose was significantly higher at final follow-up compared to start of study in responders to OIT (N = 17, difference in median tolerated dose = 126 g, p = 0.0009), in contrast to the controls (N = 13, difference in median tolerated dose = 0 g).[22] Tolerance to other cross-reactive fruits, vegetables, and nuts was reported to varying degrees (14–29%) in the OIT group by Kopac *et al.*[22] Bouvier *et al.* stated that 98% of subjects who achieved tolerance to apple were able to eat other *Rosaceae* fruits (Table 3).[21] Overall, OIT with apple appears effective in inducing tolerance to apple and some cross-reacting foods in individuals with PRFA. Level of evidence for these findings was very low according to GRADE-assessment (Table 6).

(Heat) processing

In subjects with challenge-confirmed allergy to raw hazelnut, the percentage of subjects who were completely tolerant to roasted hazelnut varied from 15 to 71% amongst the two included studies (Table 4). Symptoms to both raw or roasted hazelnut were only mild, but the median dose required to elicit symptoms with roasted hazelnut appeared higher than with raw hazelnut.[25,26]

For celery, Ballmer *et al*. found that 46% of subjects experienced no symptoms to cooked celery. No subjects (0%) tolerated celery spice. Six of 12 (50%) subjects had a moderate to severe reactions to raw celery, one of 11 (9.1%) to cooked celery and three of five (60%) to celery spice.[23] Only one case of celery allergy was examined by Bohle *et al*. and this subject had mild symptoms to raw and no symptoms to cooked celery.[24] There was insufficient information to compare dose thresholds between raw and processed celery.

Carrot was evaluated in three subjects and apple in one subject.[24] All subjects had mild symptoms to raw carrot or apple and no symptoms to cooked carrot or apple. No conclusions could be drawn regarding eliciting dose.

Overall, four studies on heat processing (mainly of celery and hazelnut) found that 15–100% of subjects with challenge-confirmed allergy to raw food experienced no symptoms to the same food when heated. GRADE-assessment resulted in a very low level of evidence for each of the evaluated outcomes (Table 6).

Hypoallergenic cultivars

As described in Table 5, the percentage of subjects who remained completely asymptomatic after the final dose was described to be significantly higher for Santana apple than for Golden Delicious or Topaz apple by Kootstra *et al*. (54% *versus* 7% *versus* 7% respectively, $p = 0.002$),[29] but did not differ significantly between Santana, Golden Delicious, Elise, and Pink Lady apple according to Vlieg-Boerstra *et al*,[30] nor between Golden Delicious and G-198/Orim according to Asero *et al*.[27] All studies evaluating allergenicity of Santana apple showed that the symptom severity score after challenge with Santana apple was significantly lower than after challenge with Golden Delicious apple in subjects with pollen-related apple allergy ($p < 0.05$).[28-30] Santana apple was also reported to be significantly less allergenic than Topaz apple in one study ($p = 0.004$).[29] Vlieg-Boerstra *et al*, who compared severity of symptoms caused by Golden Delicious apple to those caused by Santana, Elise, and Pink Lady apples, conclude that Elise is also a low allergenic apple cultivar for subjects with PRFA.[30] On comparison of G-198/Orim to Golden Delicious apple in this patient population, both cultivars were found to cause the most severe reaction equally often.[27]

Regarding the dose eliciting symptoms, Bolhaar *et al*. found that the quantities needed to provoke a reaction of equal severity were on average 30 times higher for Santana than for Golden Delicious apples ($p < 0.001$).[28] Other studies did not report on this outcome.[27,29,30]

Altogether, studies comparing low to high allergenic apple cultivars showed that Santana (and possibly Elise) apples seemed to cause milder allergic reactions than Golden Delicious apples in PRFA. The quality of evidence for the three investigated outcomes was graded as very low for the effect of this intervention.

Discussion

Overall, robust evidence regarding the effect of dietary interventions on the frequency, severity and eliciting dose of allergic reactions in subjects with PRFA is lacking. Evidence regarding the effect of specific dietary avoidance advice in this population is completely absent. Nonetheless, taking the low level of evidence into account, this systematic review of the available literature suggests that certain dietary treatments or adjustments can be beneficial for this group of patients. First of all, OIT with Golden Delicious apple seems to be effective in reducing the frequency of allergic reactions in subjects with birch pollen-related apple allergy, inducing tolerance in 63–81% of subjects. Secondly, heating of foods cross-reacting with birch or mugwort pollen appears to reduce allergenicity in subjects with PRFA, leading to complete prevention of allergic symptoms in 15–100%. Heating also possibly increases the dose threshold for symptom elicitation in pollen-related hazelnut allergic subjects. Finally, Santana and possibly Elise apples seem to cause less severe allergic reactions than Golden Delicious apples in subjects with birch pollen-related apple allergy.

Oral immunotherapy

OIT with apple was found to result in tolerance in 63%[22] and 81%[21] of subjects, a varying but high response rate. Previous studies on the effect of OIT with plant-based foods mainly focused on peanut and were not included in this review because they were performed mainly in children and without the inclusion criterion of pollen allergy.[14,15] However, these studies on peanut OIT showed that the rate of tolerance was found to range similarly to our review from 61–100%.[14,15] Sustained unresponsiveness in peanut studies, characterised by absence of symptoms to peanut despite irregular intake or prolonged avoidance, was achieved less frequently in 30–78%.[14,15] Although no evaluation of sustained unresponsiveness was performed for OIT with apple in the studies included in this review, Kopac *et al*. also suggest that tolerance may be transient, because no significant immunologic changes were observed and one subject experienced a relapse after discontinuing apple consumption during a holiday.[22]

Table 3. Summary of findings for oral immunotherapy with Golden Delicious apple

Source	**Number of subjects**	**Build-up phase completed**, *N* (%)	**Maintenance phase completed**, *N* (%)	**Frequency of achieved tolerance at final follow-up**, *N* (%)*	**Tolerated dose**	**Frequency of achieved tolerance to other birch pollen cross-reacting roods**
Bouvier *et al.*[21]	52 Active: 52 Control: NA	46/52 (88.5) Week 24; 1 non-responder; 5 drop-outs	42/52 (80.8) Week 48; 4 missings	42/52 (80.8)	No information	41/42 subjects reported tolerance to other *Rosaceae* fruits (cherries, peaches), kiwi fruit, nuts, and peanuts. One apple-tolerant subject reported being unable to consume carrots.
Kopac *et al.*[22]	40 Active: 27 Control: 13	17/27 (63.0) Week 20 (range 7–30); 5 non-responders; 5 drop-outs	17/27 (63.0) Month 8 (T8); 0 missings	**Active:** 17/27 (63.0) **Control:** 0/13 (0.0) p (active *vs* control) = 0.0001	**Active:** *Responders (N = 17):* Median tolerated dose $\Delta_{T8\text{-}T0}$: 126 (69–127) g $p(\Delta_{median\ tolerated\ dose}) = 0.0009$ *Non-responders (N = 5):* Median tolerated dose $\Delta_{T8\text{-}T0}$: 8 (0–60) g $p(\Delta_{median\ tolerated\ dose}) = NA$ **Control:** Median tolerated dose $\Delta_{T8\text{-}T0}$: 0 (24–6) g $p(\Delta_{median\ tolerated\ dose}) = NA$	Of subjects who reported symptoms to cross-reactive fruits in the active group and who completed protocol, 29% could tolerate pear where they could previously not. 27% could tolerate cherries, 23% hazelnuts, 14% walnuts, 18% peaches. In the control group 1 patient could tolerate pear where he could previously not; no other changes were observed.

Responders: subjects who successfully reached maintenance dose following oral immunotherapy (OIT) protocol. Non-responders: subjects who could not successfully reach maintenance dose despite following OIT protocol. *Intention-to-treat analyses. *NA, not available.*

Table 4. Summary of findings for (heat) processing

Source	Food and number of subjects	Frequency of NO symptoms in DBPCFC with processed food, *N (%)*	Frequency of symptoms in DBPCFC with processed Food, *N (%)*	Symptom severity in DBPCFC with raw *vs* processed food*	Eliciting dose in DBPCFC with raw *vs* processed food
Ballmer-Weber *et al.*[23]	Cooked celery: 11 Celery spice:** 5	Cooked celery: 5/11 (45.5) Celery spice: 0/5 (0)	Cooked celery: 6/11 (54.5) Celery spice: 5/5 (100.0)	Raw celery: 6× mild, 3× moderate, 3× severe Cooked celery: 5× no symptoms, 5× mild; 1× severe Celery spice: 2× mild; 3× moderate	Raw celery: 7× 0.7g; 3× 28.5g Cooked celery: 3× 0.9g, 2× 1.8g, 1× 34.5g Celery spice: 3× 0.16g, 1× 0.32g, 1× 5.85g*
Bohle *et al.*[24]	Carrot: 3 Celery: 1 Apple: 1	Cooked food: 5/5 (100.0)	Cooked food: 0/5 (0.0)	Raw food: 5× mild Cooked food: 5× no symptoms	No information
Hansen *et al.*[25]	Hazelnut: 17	Roasted hazelnut: 12/17 (70.6)	Roasted hazelnut: 5/17 (29.4)	Raw hazelnut: 17× mild Roasted hazelnut: 12× no symptoms, 5x mild	Raw hazelnut: Median dose Copenhagen (N = 10) 2g; Zurich (N = 7) 2g Roasted hazelnut: Median dose Copenhagen (N = 4) 7g; Zurich (N = 1) 5.2g *p (roasted vs raw) = NA*
Worm *et al.*[26]	Hazelnut: 82	Roasted hazelnut: 3/20 (15.0)	Roasted hazelnut: 17/20 (85.0)	Raw hazelnut: 78× mild, 4× unclear Roasted hazelnut: 17× mild	Raw hazelnut: Median dose 0.1g, range 0.01–2.0g Roasted hazelnut: Median dose 0.23g, range 0.01–10.0g *p (roasted vs raw) = 0.009*

*Mild: oral allergy symptoms, rhinitis, conjunctivitis, pruritus. Moderate: urticaria, angioedema, flush, vertigo, gastrointestinal symptoms. Severe: dyspnoea, collapse **Protein content of celery spice is 4.5 times as high as the protein content of raw celery. *DBPCFC, double-blind placebo-controlled food challenge; NA, not available.*

Table 5. Summary of findings for hypoallergenic cultivars

Source	Number of subjects	Food	Frequency of NO symptoms to highest dose in FC, *N* (%)	Symptom severity in FC with various cultivars	Dose eliciting symptoms in FC with various cultivars
Asero *et al.*[27]	7	High allergenic Golden Delicious *vs* low allergenic G-198/ Orim	GD: 0/7 (0.0) G-198/Orim: 0/7 (0.0) (*p = NA*)	GD: median VAS score 2/10; range 2–8. G-198/Orim: median VAS score 4/10; range 2–8. 2/7 patients reported more severe OAS to GD than to G-198. 2/7 patients reported more severe symptoms to G-198 than to GD. 3/7 subjects reported identical severity of symptoms to both apple cultivars.	NA, only 1 dose (15 g)
Bolhaar *et al.*[28]	5	High allergenic Golden Delicious *vs* low allergenic Santana	No information	Mean VAS score after dose 1 (5 g): Santana < GD *(p >0.05);* Mean VAS score after dose 2 (40 g): Santana < GD *(p <0.05);* Mean VAS score after dose 3 (120 g): Santana < GD *(p <0.05)*	"The quantities needed to provoke a similar VAS score were on average 30 times higher for Santana than for GD apples (p < 0.001)"
Kootstra *et al.*[29]	15	High allergenic Golden Delicious *vs* low allergenic Santana and Topaz	GD: 1/15 (6.7) Topaz: 1/15 (6.7) Santana: 8/15 (53.5) *p (Santana vs GD/Topaz) = 0.002*	Maximum VAS score after dose 1 (20 g): Santana < GD (*p = 0.017*), Santana < Topaz (*p = 0.004*)	No information
Vlieg-Boerstra *et al.*[30]	33	High allergenic Golden Delicious *vs* low allergenic Elise, Santana and Pink Lady	6–16%; no significant differences between GD, Elise, Santana and Pink Lady (*p = NA*)	Cumulative VAS score after dose 1 (15 g): Elise < Santana (*p = 0.02*), Elise < PL (*p = 0.04*), Elise < GD (*p < 0.001*); Santana < GD (*p = 0.05*)	No information

GD, Golden Delicious; FC, food challenge; PL, Pink Lady; VAS, visual analogue score; NA, not available.

Table 6. GRADE assessment

Outcome	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall GRADE
Oral immunotherapy							
Frequency of achieved tolerance to apple at final follow-up[21,22]	3	−1	−1	0	−1	0	VERY LOW
(Heat) processing							
Frequency of (NO) symptoms in DBPCFC processed food[23-26]	2	−1	−1	−1	−1	0	VERY LOW
Symptom severity in DBPCFC raw *vs* processed food[23-26]	2	−1	−1	−1	−1	0	VERY LOW
Dose eliciting symptoms in DBPCFC raw *vs* processed food[23,24,26]	2	−1	−1	−1	−1	0	VERY LOW
Consumption of hypoallergenic cultivars							
Frequency of NO symptoms to highest dose in FC[27,29,30]	2	−1	−2	0	−1	0	VERY LOW
Severity of symptoms in FC with various cultivars[27-30]	2	−1	−2	0	−1	0	VERY LOW
Dose eliciting symptoms in FC with various cultivars[28]	2	−1	−2	0	−1	0	VERY LOW

(DBPC)FC, (double-blind placebo-controlled) food challenge.

Therefore, OIT with apple in subjects with pollen-related apple allergy may be effective, but regular consumption after completion of the study is likely necessary to maintain tolerance.

Both Kopac *et al*. and Bouvier *et al*. reported that the majority of subjects with pollen-related apple allergy could consume other fruits and nuts after OIT with apple.[21,22] An explanation could be that these cross-reactive birch pollen-related foods share homologous amino acid sequences, and therefore allergenic epitopes on the surface of these homologues.[31] Desensitisation to these epitopes in apple might result in desensitisation to these epitopes in other cross-reacting foods, inducing tolerance to more foods than just apple.

(Heat) processing

The effect of heating on clinical presentation of PRFA has mainly been investigated for hazelnut and celery, and was found to eradicate symptoms in 15–71% of hazelnut and around 46% of celery allergic subjects. Furthermore, roasting of hazelnut resulted in higher dose thresholds,[25,26] and boiling of celery caused fewer moderate to severe reactions.[23] Sensitisation to the birch pollen-related PR-10 proteins, which are heat labile, explains the symptom diminishing effect of heat processing.[32] However, the effect of heating does not appear to be equivalent for all Bet v 1 homologues.[24,32] Where apple Mal d 1 undergoes a continuous unfolding process during thermal processing, carrot Dau c 1 and celery Api g 1 do not begin to change structure until higher temperatures (respectively 28 °C, 43 °C, and 50 °C).[24] Furthermore, Api g 1 returns to its native structure after cooling, where Mal d 1 and Dau c 1 do not.[24] Hazelnut Cor a 1 is reported to be heat resistant below 100 °C.[33] These findings imply that, although heating may reduce symptoms in subjects with birch PRFA, this effect differs depending on the food.

However, not only the level of heating appears to influence the allergenicity of pollen-related foods, as heating at the same temperature depleted allergenicity in some subjects but not in others. An explanation is that subjects may also be sensitised to heat stable allergens, such as lipid transfer proteins (e.g., hazelnut Cor a 8, celery Api g 2) or seed storage proteins (e.g., hazelnut Cor a 9 and 14).[23,33,34] (Co-)sensitisation to these allergens may explain why some subjects reacted to cooked celery[23] or roasted hazelnut[25,26]. In fact, Ballmer *et al*. provide support for this statement by demonstrating that not all celery allergic subjects were sensitised to the birch pollen-related Api g 1. Another explanation could be that the effect of heat processing foods in pollen-allergic subjects depends on the type of pollen sensitisation. For example, one study demonstrated that celery-mugwort sensitised subjects were IgE sensitised to heated celery, whereas celery-birch sensitised subjects were not,[35] indicating that mugwort-sensitised subjects may be more likely to react to heated celery.

Processing to spice does not appear to have the same effect as heat processing, but this was only studied for celery. All celery allergic subjects who underwent challenge with celery spice were found to be allergic to celery spice as well as to raw celery.[23] A previous *in vitro* study by Jankiewizc *et al*. also showed that it is possible to detect Api g 1, Api g 4 and celery CCD in celery spice,[17] which supports the *in vivo* findings by Ballmer-Weber *et al*. Therefore, celery spice is not safe for pollen-related celery allergic subjects.

Consumption of hypoallergenic cultivars

To date, research with regard to the effect of consumption of hypoallergenic cultivars on clinical symptoms in subjects with PRFA has focused on apple, showing that Santana apple appears to cause significantly less severe reactions than Golden Delicious apple.[28-30] These findings were later strengthened in a non-clinical setting, where around 40% of consumers with mild to moderate self-classified apple allergy reported having no symptoms to Santana apple.[36] Another apple which could be considered clinically preferable in this patient population based on the results of this review are Elise apples.[30]

However, there were also some differences between similar apple cultivar comparisons in the different studies. For example, in Kootstra *et al*. subjects reached DBPCFC final dose (±100 g) of Santana apple significantly more often than of Golden Delicious apple,[29] whereas no significant difference was found between the same cultivars in Vlieg-Boerstra *et al*. (final dose 120 g).[30] Other factors to take into consideration which may influence severity of allergic reactions to apple are season,[37] storage,[28,30,38] consumption with or without peel,[39] and intra-cultivar variation,[27] though these elements were not part of this review.

It also becomes clear that classification of apple as hypoallergenic based on SPT[28,30] or Mal d 1 content[27] does not imply equally reduced symptomatology compared to high allergenic apples like Golden Delicious. Although Santana, Topaz, Pink Lady, and Elise were all classified as low allergenic,[28,30] VAS scores of Santana were significantly lower than those of Topaz and VAS scores of Elise were significantly lower than those of Santana and Pink Lady.[29,30] Neither SPT nor Mal d 1 content seem to predict allergenicity of different apple cultivars as determined by food challenge.[40]

Strengths and limitations

In evaluating this review, the reader should remain aware of the low level of evidence due to small sample sizes, suboptimal study designs, and heterogeneity in intervention and outcome reporting between studies. The latter aspect discouraged pooling and meta-analysis. We would also like to point out that several studies had different primary aims than our review question. This meant that we had to focus on subgroups that dealt with our research question in some studies, possibly

introducing selection bias as not all characteristics of the selected subgroups were available.[23,26,27] Finally, we were unable to find any studies in which the effect of dietary avoidance advice in practice on the frequency and severity of allergic reactions was evaluated.

Nonetheless, this is the first review analysing PRFA from a dietary point of view, in which we present an overview of potentially relevant dietary interventions to aid physicians, dietitians, and nutritionists in advising and treating these patients in practice. We feel our broad research question, extensive search strategy, transparent critical appraisal, and concise presentation of study characteristics and results will allow readers to make a conscious appreciation and interpretation of the available information.

Conclusions

In conclusion, subjects with pollen-related apple allergy may benefit from OIT with apple, which may additionally reduce symptoms to cross-reactive foods. Furthermore, apple allergic patients can expect less severe reactions if they consume the hypoallergenic apple cultivars Santana or Elise. Additionally, thermal processing of causative foods in subjects with PRFA likely reduces symptoms, but the effect size may depend on the food concerned. These findings can be used to advise subjects with PRFA on their diet, taking into account that the level of evidence is low.

In the knowledge that up to 90% of pollen-sensitised individuals suffer from PRFA,[1-4] which can cause symptoms to a wide variety of fruits, nuts, and vegetables and thus deprive these individuals of valuable sources of vitamins, minerals, and fibre, more dietary intervention studies are necessary to consolidate our findings and evaluate the effect of avoidance versus allowance of causative foods, traces of causative foods and cross-reactive foods in the diet of patients with PRFA.

References

1. Bircher A, Van Melle G, Haller E, Curty B, Frei PC. IgE to food allergens are highly prevalent in patients allergic to pollens, with and without symptoms of food allergy. Clin Exp Allergy. 1994;24:367–374.
2. Flores E, Cervera L, Sanz ML, Diaz-Perales A, Fernandez J. Plant food allergy in patients with pollinosis from the Mediterranean area. Int Arch Allergy Immunol. 2012;159:346–354.
3. Osterballe M, Hansen TK, Mortz CG, Bindslev-Jensen C. The clinical relevance of sensitization to pollen-related fruits and vegetables in unselected pollen-sensitized adults. Allergy.2005;60:218–225.
4. Zuberbier T, Edenharter G, Worm M, Ehlers I, Reimann S, Hantke T, et al. Prevalence of adverse reactions to food in Germany – a population study. Allergy. 2004;59:338–345.
5. Werfel T, Asero R, Ballmer-Weber BK, Beyer K, Enrique E, Knulst AC, et al. Position paper of the eaaci: Food allergy due to immunological cross-reactions with common inhalant allergens. Allergy. 2015; 70:1079–1090.
6. Worm M, Jappe U, Kleine-Tebbe J, Schafer C, Reese I, Saloga J, et al. Food allergies resulting from immunological cross-reactivity with inhalant allergens: Guidelines from the German society for allergology and clinical immunology (dgaki), the german dermatology society (ddg), the association of German allergologists (aeda) and the society for pediatric allergology and environmental medicine (gpa). Allergo J Int. 2014;23:1–16.
7. Kumar R. Epidemiology and risk factors for the development of food allergy. Pediatr Ann. 2008;37: 552–558.
8. Lack G. Epidemiologic risks for food allergy. J Allergy Clin Immunol. 2008;121:1331–1336.
9. Madsen C. Prevalence of food allergy: An overview. Proc Nutr Soc. 2005;64:413–417.
10. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. Eaaci food allergy and anaphylaxis guidelines: Diagnosis and management of food allergy. Allergy. 2014;69:1008–1025.
11. Price A, Ramachandran S, Smith GP, Stevenson ML, Pomeranz MK, Cohen DE. Oral allergy syndrome (pollen-food allergy syndrome). Dermatitis. 2015;26:78–88.
12. Ma S, Sicherer SH, Nowak-Wegrzyn A. A survey on the management of pollen-food allergy syndrome in allergy practices. J Allergy Clin Immunol. 2003;112:784–788.
13. Kinaciyan T, Nagl B, Faustmann S, Frommlet F, Kopp S, Wolkersdorfer M, et al. Efficacy and safety of 4 months of sublingual immunotherapy with recombinant mal d 1 and bet v 1 in patients with birch pollen-related apple allergy. J Allergy Clin Immunol. 2018;141:1002–1008.
14. Gernez Y, Nowak-Wegrzyn A. Immunotherapy for food allergy: Are we there yet? J Allergy Clin Immunol Pract. 2017;5:250–272.
15. Yee CS, Rachid R. The heterogeneity of oral immunotherapy clinical trials: Implications and future directions. Curr Allergy Asthma Rep. 2016;16:25.
16. Asarnoj A, Moverare R, Ostblom E, Poorafshar M, Lilja G, Hedlin G, et al. IgE to peanut allergen components: Relation to peanut symptoms and pollen sensitization in 8-year-olds. Allergy. 2010;65: 1189–1195.
17. Jankiewicz A, Baltes W, Bögl W, Dehne L, Jamin A, Hoffmann A, et al. Influence of food processing on the immunochemical stability of celery allergens. J Sci Food Agric. 1997;75:359–370.
18. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The prisma statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. BMJ. 2009;339:b2700.
19. Sterne JA, Hernan MA, Reeves BC, Savovic J, Berkman ND, Viswanathan M, et al. Robins-i: A tool for assessing risk of bias in non-randomised studies of interventions. BMJ. 2016;355:i4919.
20. How to grade the quality of the evidence. Available online: https://www.google.com.tw/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=2ahUKEwi8pNuf5P3dAhXFAIgKHZN9B0EQFjAAegQIBRAC&url=https%3A%2F%2Fcc.cochrane.org%2Fsites%2Fcc.cochrane.org%2Ffiles%2Fpublic%2Fuploads%2Fhow_to_grade.pdf&usg=AOvVaw2juZxoBHf5FSRuWAiHVkLa (accessed on 8 Aug 2018).

21. Bouvier M, Van Der Brempt X, Nosbaum A, Codier JM, Cherih C, Frappaz A, et al. L'induction de tolérance orale dans lállergie aux rosacées. Revue Française d'Allergologie. 2014;54:127–133.
22. Kopac P, Rudin M, Gentinetta T, Gerber R, Pichler C, Hausmann O, et al. Continuous apple consumption induces oral tolerance in birch-pollen-associated apple allergy. Allergy. 2012;67:280–285.
23. Ballmer-Weber BK, Hoffmann A, Wuthrich B, Luttkopf D, Pompei C, Wangorsch A, et al. Influence of food processing on the allergenicity of celery: DBPCFC with celery spice and cooked celery in patients with celery allergy. Allergy. 2002;57:228–235.
24. Bohle B, Zwolfer B, Heratizadeh A, Jahn-Schmid B, Antonia YD, Alter M, et al. Cooking birch pollen-related food: Divergent consequences for IgE- and t cell-mediated reactivity in vitro and in vivo. J Allergy Clin Immunol. 2006;118:242-249.
25. Hansen KS, Ballmer-Weber BK, Luttkopf D, Skov PS, Wuthrich B, Bindslev-Jensen C, et al. Roasted hazelnuts--allergenic activity evaluated by double-blind, placebo-controlled food challenge. Allergy. 2003;58:132–138.
26. Worm M, Hompes S, Fiedler EM, Illner AK, Zuberbier T, Vieths S. Impact of native, heat-processed and encapsulated hazelnuts on the allergic response in hazelnut-allergic patients. Clin Exp Allergy. 2009;39:159–166.
27. Asero R, Marzban G, Martinelli A, Zaccarini M, Machado ML. Search for low-allergenic apple cultivars for birch-pollen-allergic patients: Is there a correlation between in vitro assays and patient response? Eur Ann Allergy Clin Immunol. 200;38:94–98.
28. Bolhaar ST, van de Weg WE, van Ree R, Gonzalez-Mancebo E, Zuidmeer L, Bruijnzeel-Koomen CA, et al. In vivo assessment with prick-to-prick testing and double-blind, placebo-controlled food challenge of allergenicity of apple cultivars. J Allergy Clin Immunol. 2005;116:1080–1086.
29. Kootstra HS, Vlieg-Boerstra BJ, Dubois AE. Assessment of the reduced allergenic properties of the Santana apple. Ann Allergy Asthma Immunol. 2007;99:522–525.
30. Vlieg-Boerstra BJ, van de Weg WE, van der Heide S, Kerkhof M, Arens P, Heijerman-Peppelman G, et al. Identification of low allergenic apple cultivars using skin prick tests and oral food challenges. Allergy. 2011;66:491–498.
31. Neudecker P, Schweimer K, Nerkamp J, Scheurer S, Vieths S, Sticht H, et al. Allergic cross-reactivity made visible: Solution structure of the major cherry allergen pru av 1. J. Biol Chem. 2001;276:22756–22763.
32. Mills EN, Sancho AI, Rigby NM, Jenkins JA, Mackie AR. Impact of food processing on the structural and allergenic properties of food allergens. Mol Nutr Food Res. 2009;53:963–969.
33. Verhoeckx KC, Vissers YM, Baumert JL, Faludi R, Feys M, Flanagan S, et al. Food processing and allergenicity. Food Chem Toxicol. 2015;80:223–240.
34. Datema MR, Zuidmeer-Jongejan L, Asero R, Barreales L, Belohlavkova S, de Blay F, et al. Hazelnut allergy across Europe dissected molecularly: A europrevall outpatient clinic survey. J Allergy Clin Immunol. 2015;136:382–391.
35. Egger M, Mutschlechner S, Wopfner N, Gadermaier G, Briza P, Ferreira F, et al. Pollen-food syndromes associated with weed pollinosis: An update from the molecular point of view. Allergy. 2006;6:461–476.
36. Maas vd MP, Schen MF. Development of a protocol that allows safe consumption of the hypoallergenic apple cultivar Santana. Acta Hortic. 2009, 549–552.
37. Geroldinger-Simic M, Zelniker T, Aberer W, Ebner C, Egger C, Greiderer A, et al. Birch pollen-related food allergy: Clinical aspects and the role of allergen-specific IgEand IgG4 antibodies. J Allergy Clin Immunol. 2011;127:616–622.
38. Kiewning D, Schmitz-Eiberger M. Effects of long-term storage on mal d 1 content of four apple cultivars with initial low mal d 1 content. J Sci Food Agric. 2014;94:798–802.
39. Fernandez-Rivas M, Cuevas M. Peels of rosaceae fruits have a higher allergenicity than pulps. Clin Exp Allergy 1999;29:1239–1247.
40. Nybom H, Cervin-Hoberg C, Andersson M. Oral challenges with four apple cultivars result in significant differences in oral allergy symptoms. Int Arch Allergy Immunol. 2013;161:258–264.

Supplemental files

Search strategy for PubMed search (performed 6 July 2018)

DOMAIN: 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] OR poaceae [MeSH Terms] OR poaceae* [Title/Abstract] OR grass* [Title/Abstract] OR timothy* [Title/Abstract] OR Artemisia [MeSH Terms] OR artemisia* [Title/Abstract] OR ambrosia [MeSH Terms] OR ambrosia* [Title/Abstract] OR mugwort* [Title/Abstract] OR ragweed* [Title/Abstract] OR plant weeds [MeSH Terms] OR weed* [Title/Abstract]

7. 5 AND 6
8. oral allergy syndrom* [Title/Abstract]) OR pollen food syndrom* [Title/Abstract]
9. 7 OR 8

DETERMINANTS: Oral immunotherapy, (heat) processing, hypoallergenic cultivars, dietary avoidance

10. Immunotherapy [MeSH Terms] OR immunotherap* [Title/Abstract]
11. Oral [Title/Abstract] AND tolerance [Title/Abstract] AND induc* [Title/Abstract]
12. 10 OR 11
13. Heating [MeSH Terms] OR heat* [Title/Abstract] OR cooking [MeSH Terms] OR cook* [Title/Abstract] OR roast* [Title/Abstract] OR baked [Title/Abstract] OR baking [Title/Abstract] OR microwav* [Title/Abstract] OR pasteuriz* [Title/Abstract] OR pasteuris* [Title/Abstract] OR process* [Title/Abstract] OR dehydrat* [Title/Abstract] OR dried [Title/Abstract] OR spice* [Title/Abstract] OR herb* [Title/Abstract]
14. hypoallergen* [Title/Abstract] OR hypo allergen* [Title/Abstract] OR low allergen* [Title/Abstract] OR reduced allergen* [Title/Abstract] OR cultiv* [Title/Abstract] OR variet* [Title/Abstract]
15. diet [Title/Abstract]) OR diets [Title/Abstract] OR dietary [Title/Abstract] OR avoid* [Title/Abstract] or trace [Title/Abstract] OR traces [Title/Abstract]
16. 12 OR 13 OR 14 OR 15
17. 9 AND 1

Chapter 10

General discussion

Thorough exploration of data collected in a standardised manner all across Europe, mostly during the pan-European EuroPrevall project, has brought us a little closer to unravelling the enigma that is food allergy (FA). FA prevalence patterns, prediction and patient profiles were the focus of this thesis. This general discussion will summarise and discuss the main findings, corresponding (clinical) implications and considerations for future research.

MAIN FINDINGS

I. Food allergy in the general population: prevalence patterns and potential risk factors

- In both school-age children and adults, FA prevalence estimates show considerable geographical variation across Europe. Prevalence of FA based on self-report substantially overestimates prevalence of clinically manifest FA. - **Chapter 2 and 3**
- In school-age children across Europe, prevalence of self-reported FA ranges from 7% in Greece to 25% in Poland; prevalence of food sensitisation (FS) from 11% in Iceland to 29% in Switzerland; and prevalence of probable FA from 2% in Iceland to 6% in Poland. **- Chapter 2**
- In adults across Europe, a previous EuroPrevall study showed that prevalence of self-reported FA ranges from less than 1% in Lithuania to 19% in Spain and prevalence of FS from 7% in Iceland to 24% in Switzerland.[1] Prevalence of probable FA in adults is lowest in Greece at less than 1%, and highest in Switzerland at 6%. **- Chapter 3**
- The foods most often responsible for FS and probable FA differ depending on the country. Geographical patterns of prevalence and causative foods seem related to local pollen and food exposure. Food sensitisation to plant source foods caused by cross-reactivity with birch pollen is likely responsible for the high prevalence of FA to hazelnut, apple, peach, kiwi, celery and carrot in birch-endemic Northern and Central Europe (Switzerland, the Netherlands, Poland and Lithuania). The absence of birch pollen in combination with local dietary preferences may explain why other plant source foods dominate alongside peach and kiwi in the Mediterranean regions, such as melon, banana, walnut, peanut, lentils and sunflower seeds. Consumption of seafood is highest in the most Northern and most Southern parts of Europe, which could be a reason why allergy to fish and shrimp is mostly observed in Spain, Greece and Iceland. **- Chapter 2 and 3**
- Dog ownership in early childhood is inversely associated with FS in later childhood. Other early-life environmental exposures appear to have a more limited impact on occurrence of FS in childhood and/or adulthood, though preventative tendencies are observed for certain determinants, including

having multiple (older) siblings, day care attendance, bedroom sharing, growing up in a farm environment, and early introduction of solid foods. **- Chapter 4**

II. Food allergy in the presenting patient: prediction, patient profiles and pollen-related food allergy

- Information available from patient history can accurately predict IgE sensitisation corresponding to a food specific case history (i.e. probable FA), especially for plant source culprit foods, and especially in adults. In both school-age children and adults reporting adverse reactions to (primarily plant source) foods, oral allergy symptoms and allergic rhinitis comorbidity are strongly associated with presence, and gastrointestinal symptoms with absence of probable FA. **- Chapter 5**
- Besides patient history and routine extract-based diagnostic tests, component-resolved diagnostics (CRD) are becoming increasingly important in the FA diagnostic workup. However, in Dutch adult individuals with suspected **hazelnut** allergy, neither IgE to hazelnut extract nor IgE to hazelnut components can accurately discriminate between presence and absence of hazelnut allergy, as determined by double-blind placebo-controlled food challenge (DBPCFC). **- Chapter 6**
- Clinical background determinants are most valuable for predicting severity of probable **peanut** and **walnut** allergy in an individual patient. Neither extract- nor component-based testing can accurately discriminate between mild-to-moderate and severe probable walnut or peanut allergy in (mostly adult) European patients. For **walnut**, combining data from clinical background with data from extract-based testing and CRD leads to improved prediction of severity of probable walnut allergy. For **peanut**, extract- and component-based tests were found to have limited predictive value in addition to clinical background determinants. **- Chapter 7 and 8**
- Pollen-related FA (PRFA) is common and thought to become more prevalent. Oral immunotherapy, heat processing, and consumption of hypoallergenic cultivars may diminish or completely prevent allergic reactions in some but not all subjects with PRFA. **- Chapter 9**

I. FOOD ALLERGY IN THE GENERAL POPULATION

FA did not truly capture the attention of the medical world until the 1980s, when the first publications on fatal anaphylactic reactions to food appeared.[2-4] Since then, the evidence base for assessment and management of FA has expanded exponentially.[5] Unsurprisingly, the increased awareness of FA amongst physicians and investigators in recent years transferred to the general population.[6] Population-wide prevention strategies, such as legislation on precautionary allergen labelling and recommendations regarding timing of introduction of allergenic foods into infant diets, gained particular interest.[7-11] FA became a hot topic. Nowadays, more and more individuals identify themselves or their children as food allergic, resulting in increased FA prevalence estimates, but undoubtedly also in misclassification.

The consistently collected EuroPrevall data provided a unique opportunity to validly estimate and compare FA prevalence estimates according to various relevant outcome definitions in the general population across major climatic and cultural regions of Europe, as summarised from ***Chapter 2 and 3*** in ***Figure 1***. In ***Chapter 4***, contribution of (mainly early-life environmental) determinants to prediction of FS in Europe's general population were subsequently evaluated.

Part I of this general discussion will revisit some of the main findings in ***Chapter 2 to 4*** of this thesis, and is structured as follows:

1. Self-reported FA greatly overestimates clinically manifest FA
2. FS greatly overestimates clinically manifest FA
3. FA prevalence in school-age children *versus* FA prevalence in adults
4. Substantial variation in prevalence of FA across Europe
5. Prevalence of European FA in a global context
6. Sources of FA prevalence variation
   - 6.1 Food exposure
     - 6.1.1 Frequency and quantity of consumption
     - 6.1.2 Age of introduction
     - 6.1.3 Route of exposure
     - 6.1.4 Food processing
     - 6.1.5 Food exposure and geographical variation of FA prevalence
   - 6.2 Pollen exposure
   - 6.3 Microbial exposure

1. Self-reported FA greatly overestimates clinically manifest FA

The EuroPrevall community surveys focused particularly on 24 foods commonly implicated in FA or frequently consumed in participating countries (the so-called EuroPrevall *priority foods*): hen's egg, cow's milk, fish, shrimp, hazelnut, walnut, peach, apple, kiwi, melon, banana, tomato, celery, carrot, peanut, soy, lentils, wheat, buckwheat, corn, sesame seed, mustard seed, sunflower seed, and poppy seed. Prevalence estimates of self-reported FA (to any food or to priority foods) were found to range from 7 to 48% in school-age children and from <1 to 37% in adults across Europe (***Figure 1***). These estimates are comparable to those previously obtained from systematic review of European literature, according to which prevalence of self-reported FA is as high as 42% in children and 35% in adults in some parts of Europe.[12]

Overall, less than 20% of children and adults with a food-specific case history had corresponding IgE sensitisation, yielding prevalence estimates of probable FA ranging from 2% to 6% in children and from less than 1% to 6% in adults (***Figure 1***). The only previous European prevalence estimates of probable FA defined as symptoms plus positive serology were obtained from Germany, where the estimates of just under 5% for children and just over 2% for adults fit well within our EuroPrevall prevalence ranges.[12-14]

Prevalence of FA as diagnosed by the diagnostic gold standard, (double-blind placebo-controlled) food challenge, is lower still. Literature reports prevalence estimates of 0.4 to 4% for children and 0.1 to 3% for adults.[12] The low DBPCFC participation rate in the EuroPrevall community surveys prevented reliable estimation of challenge-confirmed FA prevalence. However, the observation that FA was confirmed in 73% of adults who did agree to undergo DBPCFC, suggests a plausible population prevalence of 0.2 to 4% for challenge-confirmed FA in European adults, which is in line with literature.[12]

Our findings confirm considerable overestimation of clinically manifest FA based on self-reported FA,[12, 15] presumably mostly due to lay perception of allergy associated with any adverse reaction or aversion to food.[16]

Clinical implications

- Health care professionals should realise that the majority of subjects reporting food adverse reactions are not food allergic, but that the prevalence of probable FA still reaches an impressive 6% in some European regions.

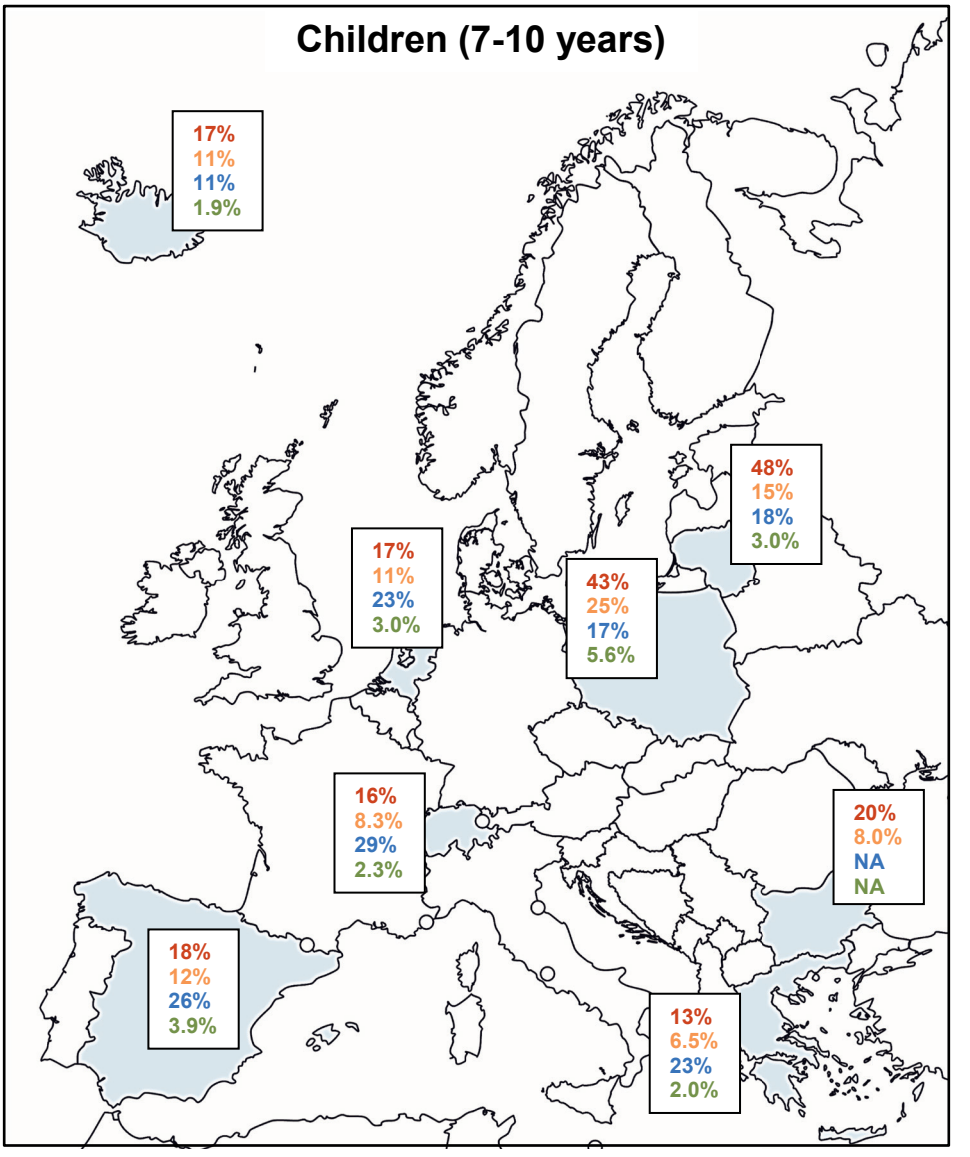

Figure 1A. Prevalence of FA in children across Europe
Self-reported food allergy to any food – **self-reported food allergy to priority food** – **food sensitisation** – **probable food allergy (complete case analysis)**. Centres: Reykjavik (Iceland), Utrecht (Netherlands), Lodz (Poland), Vilnius (Lithuania), Zurich (Switzerland), Madrid (Spain), Sofia (Bulgaria), Athens (Greece). *NA, not available.*

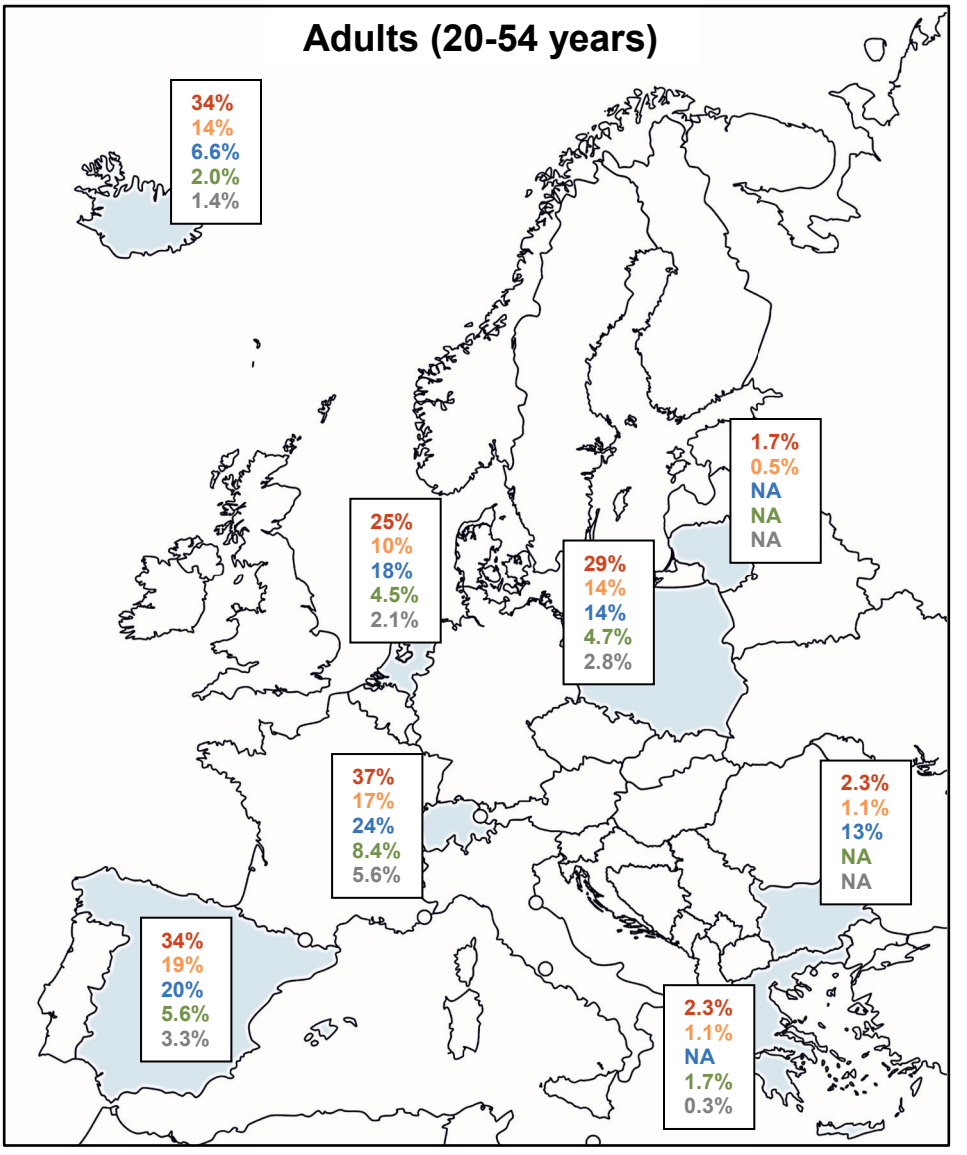

Figure 1B. Prevalence of FA in adults across Europe
Self-reported food allergy to any food – **self-reported food allergy to priority food** – **food sensitisation** – **probable food allergy (complete case analysis)** – **probable food allergy (multiple imputation)**. Centres: Reykjavik (Iceland), Utrecht (Netherlands), Lodz (Poland), Vilnius (Lithuania), Zurich (Switzerland), Madrid (Spain), Sofia (Bulgaria), Athens (Greece). Data on FS in adults were extracted from Burney et al.[1] *NA, not available.*

2. FS greatly overestimates clinically manifest FA

A remarkable percentage of EuroPrevall children and adults were found to be sensitised to at least one priority food. In ***Chapter 2 and 3*** we showed that IgE antibodies were detected in 11 to 29% of children and 7 to 24% of adults (***Figure 1***). However, the much lower prevalence estimates of probable FA reinforce that FS does not invariably lead to FA. A breakdown in the process of oral tolerance induction leads to the immune response characteristic of FS and clinically manifest FA.[17, 18] After initial ingestion and digestion of a food, food proteins and protein fragments are transported across the epithelium from the gut lumen to the gut mucosa and internalised by dendritic cells, which move to mesenteric lymph nodes in order to present the food antigens to naïve $CD4^+$ T cells on MHC class II molecules.[17, 19] After antigen presentation, naïve CD4+ T cells differentiate into one of several lineages of T helper cell subtypes. Tolerance is linked to generation of anergic regulatory T (Treg) cells in the gut lymphoid tissue, whereas allergic sensitisation follows generation of T_H2 cells.[17, 19-24] T_H2 cells secrete cytokines, such as IL-4, IL-5 and IL-13, which stimulate allergen-specific B cells to class-switch and produce IgE antibodies that bind to receptors (FcεRI) on mast cells and basophils. In other words, food-specific IgE antibodies "arm" allergic effector cells (sensitisation phase). Alternative routes of sensitisation, via the skin or the respiratory tract, are topics of current debate, and will be discussed later in subsection 6.1.3. Upon subsequent exposure to the same food antigen, cross-linking of receptor-bound IgE antibodies on these effector cells causes degranulation (elicitation phase). Inflammatory mediators like histamine lead to symptoms of clinical FA. Whether FS translates into clinical FA upon food exposure, depends on successful IgE cross-linking on mast cells and basophils, for sufficient duration (>100 seconds) and at sufficient sites (>100 cross-links per effector cell).[25] In order to achieve cross-linking, at least two epitopes on the surface of the culprit allergen or allergen aggregates must be recognised by the sensitised individual's FcεRI-bound IgE.[26, 27] Several non-mutually exclusive hypotheses are available for the discrepancy between sensitisation and clinically manifest FA.

To start, individuals with higher levels of specific IgE may recognise a broader array of allergen epitopes, and therefore be more likely to have an allergic reaction.[26, 27] Increased level of food-specific IgE has been found to correlate with an increased likelihood of a reaction upon ingestion for certain foods.[28, 29] Some studies suggest that certain antibody isotypes, like IgG_4, can counteract symptom induction through IgE.[30] Two mechanisms are proposed.[31] First of all, IgG_4 can bind to and block the IgE epitope on the allergen, preventing cross-linking of receptor-bound IgE.[31] Second, mixed IgE/IgG_4-receptor cross-linking can inhibit effector cell activation, in which case IgE binds to an FcεRI receptor and IgG_4 binds to an FcγRIIb receptor on the surface of the effector cell.[31] Studies have found that levels of peanut-specific IgG_4 and ratios of peanut-specific IgG_4/peanut-specific IgE are greater in peanut tolerant than peanut allergic subjects.[29, 32] Another important source of clinically irrelevant FS

is IgE against cross-reactive carbohydrate determinants (CCD), which may be the most widely occurring IgE epitopes, and are detected in 5-10% of non-allergic subjects and 70% of subjects with multiple pollen sensitisation.[33] It is currently not known why certain people develop anti-CCD IgE, nor why such sensitisation is clinically insignificant.[34-36] Investigators remark that low binding affinity of anti-CCD IgE is not the reason, and again speculate on an inhibitory role of IgG.[37] It is also worth noting that digestion and processing can affect the likelihood of a food allergic reaction occurring in a sensitised individual by altering existing or creating new epitopes.[38-40] Food processing will be further discussed in section 6.1.4.

In addition, allergen-independent host-specific factors affect elicitation of clinical symptoms in an IgE-sensitised subject. Such host-specific factors include, for instance, the degree of activation of intracellular signalling pathways, responsible for the synthesis and metabolism of basophil and mast cell release products, upon successful IgE-crosslinking.[41, 42] For example, mutation, deletion or blockade of key FcεRI signalling kinase *Syk* has been associated with reduced effector cell degranulation.[43-45] Furthermore, some individuals have a better intrinsic ability to compensate for secreted mediators.[46-48] Hosts who are less able to metabolise inflammatory mediators generated during food allergic reactions, such as platelet activating factor (PAF) or bradykinin,[48-50] may be more likely to have (severe) allergic reactions. For instance, (basal) levels of PAF-acetyl hydrolase (AH) and certain PAF-AH polymorphisms have been found to correlate inversely with severity of allergic reactions.[46, 47, 49, 51-53] Each of these factors is dependent on gene and protein expression as regulated by various genetic polymorphisms, transcription control mechanisms, and epigenetics. [54, 55]

Overall, clinical manifestation of FA is the result of more than just presence of specific IgE. There is a complex interplay between allergen-, antibody- and host-specific factors, which is not yet fully understood.

Clinical implications

- FS is a prerequisite for, but does not invariably lead to FA.
- Factors which may influence the translation of FS to FA in a specific patient include factors affecting antigen recognition (e.g. degree of food digestion and processing), factors affecting successful IgE cross-linking (e.g. level of specific IgE, level of IgG_4, recognition of CCD), and host-specific factors determining the degree of effector cell degranulation and the host's response to effector cell degranulation (e.g. polymorphisms in *Syk* or levels of *PAF-AH*).

3. FA prevalence in school-age children *versus* FA prevalence in adults

In ***Chapter 2*** and ***Figure 2***, we respectively described and depict how patterns of FS in school-age children correspond with transition from early childhood (commonly occurring sensitisation to cow's milk and hen's egg, and mainly primary sensitisation to plant source foods) to adulthood (mainly sensitisation to plant source foods based on cross-reactivity with pollen).[12] In ***Chapter 4***, we observed that increasing age in adults is inversely associated with FS, similarly to other cross-sectional studies.[56-58] Upon comparison of findings in children and adults in ***Chapter 2*** and ***Figure 1***, it became clear that prevalence of FS is higher in children than adults in all countries where estimates for both age groups were available, whereas prevalence of probable FA was either higher in adults or similar in both children and adults (***Figure 1***). In line with our findings, a systematic review of literature on prevalence of FA across Europe also found that overall prevalence of FS is higher among children than among adults, and that prevalence estimates of probable FA and of challenge-confirmed FA are more comparable between children and adults.[12]

Prevalence is determined by the balance between incidence and resolution rates.[59] Two explanations ought to be considered for the higher prevalence of FS in school-age children compared with adults. First of all, it seems likely that there is an increase in incidence of FS over time, as suggested by several other studies. [60-63] As the EuroPrevall paediatric cohort stems from a later childhood era than the adult cohort, an increase in incidence of FS over time could explain the higher prevalence of FS in children than in adults. Secondly, it is important to explore the possibility of FS resolving as subjects grow older. For most foods, FS is thought to persist, but high rates of resolution of cow's milk and hen's egg sensitisation have been described, not only during early childhood when the immune system is most adaptive, but also between the ages of 12 and 18 years.[64-66] This is in accordance with the EuroPrevall data, in which the largest discrepancies in prevalence of FS between children and adults were found for milk (4-15% *vs* 0-2%) and egg (3-7% *vs* 0-1%).

Based on the reasoning in the preceding paragraph, one would expect prevalence of probable FA to be higher in children than in adults as well. Indeed, prevalence of probable FA to milk and egg was much higher in children than in adults in all participating centres. However, overall prevalence of probable FA was higher in adults than in children in Switzerland, the Netherlands, and Spain (>1.5% higher), and similar between children and adults in Poland, Iceland and Greece (<1% difference). Taking into account that primary sensitisation to plant source foods was relatively more common in children than in adults (***Figure 2***), a likely explanation for the comparable overall probable FA prevalence estimates in children and adults is that FS based on cross-reactivity with pollen is more likely to be clinically relevant in the adults.

The epitope repertoire recognised by the specific IgE antibodies of individual patients determines the degree of birch pollen-related food cross-reactivity and the likelihood of successful IgE-crosslinking leading to an allergic reaction.[67, 68] It is conceivable that an individual patient's epitope repertoire expands with age, along with the likelihood of clinical manifestation of pollen-related FA. Support for the theory that cross-reactivity with pollen is more likely to be clinically relevant in adults was also found in ***Chapter 5***, where we explored how sensitisation to major birch pollen allergen Bet v 1 affected the relevance of certain features of patient history for predicting probable FA. In adults, there was a statistically significant interaction between reporting oral allergy symptoms (OAS) and Bet v 1 sensitisation. The odds ratios (ORs) of allergic rhinitis (AR) and OAS were attenuated by addition of Bet v 1 to the model. In children, there was no statistically significant interaction between reporting of OAS and Bet v 1 sensitisation, but the OR of AR was attenuated by addition of Bet v 1. These observations suggest that school-age children are sensitised to birch pollen (which causes AR), and probably have cross-reactive FS, but that birch-pollen related FA (presenting as OAS) is not yet as common an occurrence in this age group as in adults.

Taken together, increasing prevalence of cross-reactive FS over time, alongside resolution of milk and egg sensitisation with age, could explain why FS occurs more in the paediatric than in the adult population, but probable FA does not (yet). School-age children may still acquire symptoms at a later stage, suggesting prevalence of probable FA in adults may be set to rise in the future.

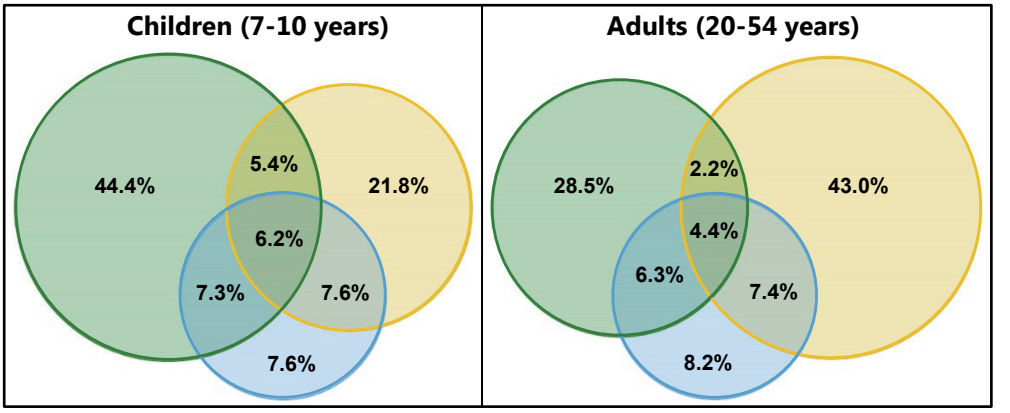

Figure 2. Cross-reactivity to plant source foods in European children
Primary plant source food sensitisation PR-10 cross-reactivity Profilin/CCD cross-reactivity

Clinical implications

- Animal source FA, plant source FA through primary (non-cross-reactive) FS, and pollen-related FA, all occur frequently in European school-age children. Plant source FA dominates in European adults; pollen-related FA appears to be particularly prominent.
- Prevalence differences between children and adults suggest a rise in prevalence of (cross-reactive) FS over time, which may lead to an increase in prevalence of FA in the future.

4. Substantial variation in prevalence of FA across Europe

The wide ranges of prevalence estimates for self-reported FA, FS and probable FA across Europe reveal a remarkable degree of geographical variation, as shown in ***Figure 1***. Geographical variation in prevalence is undoubtedly influenced by local food, pollen and possibly microbial exposure, which will be discussed extensively in section 6, after first recapping some factual data on prevalence patterns observed across Europe (this section) and across the world (section 5).

Self-reported FA to priority foods was relatively uncommon in South-Eastern Europe (Greece and Bulgaria) in both children and adults (***Figure 1***). Much higher prevalence estimates of self-reported FA were observed further to the North (particularly in Poland and Lithuania for children) and West (particularly in Spain and Switzerland for adults). Regarding FS, countries could be ranked according to the same order of prevalence in both children and adults in all countries where estimates for both age groups were available. The lowest prevalence estimates of FS were observed in Iceland, followed by Poland, the Netherlands, Spain and Switzerland in ascending order. Ultimately, probable FA was found to be least common in Greece and Iceland, both in children and adults, and most common in Polish children and in Swiss adults.

As discussed in ***Chapter 2 and 3***, the foods most often responsible for probable FA also differed per region. In Central and Northern Europe (Switzerland, the Netherlands, Poland and Lithuania), hazelnut, apple, peach, kiwi, celery and carrot were particularly common causes of probable FA in either children and adults. Other plant source foods were found to dominate alongside peach and kiwi in Mediterranean regions, such as melon, banana, walnut, peanut, lentils and sunflower seeds. Animal source foods cow's milk and hen's egg were prominent sources of probable FA in children throughout Europe, but were rarely the culprit foods in adults. Probable FA to fish or shrimp was mostly observed in Spain, Greece and Iceland.

5. Prevalence of FA in a global context

Prevalence data for FA in school-age children and adults from the general population outside Europe are scarce, and are mostly based on self-report.[69-75] Most prevalence estimates of self-reported FA outside Europe show overlap with the prevalence estimates of self-reported FA to priority foods in the EuroPrevall study; 7-25% in school-age children and <1-19% in adults. In North-America, prevalence estimates of self-reported FA range from 3 to 7% in children of all ages,[76-80] and from 8 to 12% in adults.[76, 80-82] In Central and South-America, studies from Mexico, Colombia and Brazil found self-reported FA to exist in 3-13% of children (school-age and other)[83, 84] and 11-15% of adults.[73, 84, 85] In Africa, prevalence of self-reported FA is estimated around 11% in school-age children from Ghana,[86] and 17-19% in adults from Tanzania and Mozambique.[74, 87, 88] More extensive data are available from Asia and Oceania. Self-reported FA is reported for 2-17% of children (school-age and other) from India, Korea, Japan, Taiwan, Hong Kong and China.[89-92] As many as 38% of school-age children from Tomsk, in the Asian part of Russia, were found to have self-reported FA.[89] Self-reported FA is found in 6-19% of adults from Taiwan and India,[91, 93] and in 13-19% of adults from New Zealand and Australia.[82]

The Asian counterpart of the EuroPrevall study, the INCO study, also provided prevalence estimates of probable FA, which ranged from 0.1 to 2% in school-age children from India, China, Hong Kong and the Asian part of Russia;[89] and was found to be 1% in adults from India.[93] The estimates of probable FA in the INCO study (0.1-2%) were much lower than those in the EuroPrevall study (***Figure 1***). This is particularly interesting in the context of the observation that FS was equally or more common in the Asian countries (7-27%) than in the European countries (7-29%), even with the cutoff for positive sensitisation set at 0.7 kU_A/L in the INCO study and at 0.35 kU_A/L in the EuroPrevall study.[89, 93] This apparent contradiction is still largely unexplained, but potential influence of parasitic infections on FS and FA in developing countries will be discussed in section 6.3.

The SchoolNuts study in Australia found prevalence of FA confirmed by open food challenge to reach almost 5% in children aged 10 to 14 years,[94] which seems high compared to prevalence estimates of challenge-confirmed FA for school-age children in Europe (0.4-4%).[12] Besides the possible effects of route of food exposure (section 6.1) and microbial exposure (section 6.3), investigators speculate on the influence of immigration from Asia and vitamin D insufficiency.[95] Parental migration from Asia to Australia was found to be a risk factor for (pea)nut allergy in the next generation.[95, 96] Vitamin D insufficiency was associated with increased challenge-proven FA in Australian infants.[97] In reality, the reasons for the high Australian prevalence are unknown.

Overall, prevalence estimates of self-reported FA from all over the world show overlap, but prevalence of clinically manifest FA seems to be more common in Westernised countries (particularly Australia) than in developing countries.

It is difficult to make inter-continental comparisons regarding the most commonly implicated foods, because each study focuses on different foods. Cow's milk, hen's egg, fish, shrimp, peanut, wheat and soy are mentioned as top causative foods in studies from all continents.[70, 71] Tree nuts are mainly reported in studies from the most Westernised countries, including Europe as seen in ***Chapter 2 and 3***, North-America,[76, 80, 81] Australia,[94] and the most Western parts of Asia (Tomsk in Russia).[89] Fruits and vegetables are also reported in all regions, but different types in each region, such as apple, peach, kiwi, celery and carrot in Europe (Chapter 2 and 3), but mango in Asia (Taiwan)[91] and South-America (Mexico),[83] and pineapple in Africa (Ghana).[86] Sesame is a frequently implicated food in Israel,[98] beans and corn in South-America,[71, 83] and buckwheat in Korea.[90] Certain foods are only consumed in very specific regions. As a result, bird's nest soup is only reported as relevant causative food in Singapore,[99] and okra and Mopane worms in some regions of Africa,[74] but not elsewhere in the world.

6. Sources of prevalence variation

Geographical variation in prevalence of FA and changes in prevalence of FA over time suggest environmental influences,[100] of which food, pollen and microbial exposure are essential determinants to consider.[17-19, 23, 24, 101-105]

6.1. Food exposure

Exposure to specific foods depends on local dietary habits. In ***Chapter 2 and 3***, we described how occurrence of probable FA to fish and shrimp in both children and adults was (relatively) most likely in Spain, Greece and Iceland of the EuroPrevall countries, where seafood consumption is highest.[106] In contrast, another study reported a 10-fold higher prevalence of peanut allergy among Jewish children from the United Kingdom than among those from Israel, and that peanut consumption was much lower in the UK than in Israel.[107] In India, high prevalence of sensitisation was found for commonly consumed foods sesame seed and lentil, but also for uncommonly consumed shrimp.[93] In France, André *et al.* observed that changes in consumption frequency over time were differently associated with incidence of sensitisation depending on food: increased consumption of milk and dairy products was accompanied by a decreased incidence of sensitisation; of rice and wheat by an increased incidence of sensitisation; and of crab, peanuts and celery by an unchanged incidence of sensitisation.[108] Such observations raise the issue as to how consumption affects the likelihood of FS and FA. In relation to this topic, the following subsections will discuss how frequency and quantity of consumption, age of introduction, route of exposure and food processing may play a role. The final

paragraph will summarise how food exposure seems to affect geographical prevalence variation.

6.1.1. Frequency and quantity of consumption

A relevant question is whether the frequency with which an individual consumes a food directly affects likelihood of FS and FA. ***Figure 3*** shows that there was no correlation between frequency of food consumption and frequency of sensitisation for the majority of foods in EuroPrevall school-age children and adults. If present, the prevailing trend was one of a decrease in sensitisation as consumption frequency increases. Multivariable logistic regression analysis, adjusted for centre and preventative avoidance, showed that the inverse association between frequency of consumption and sensitisation was statistically significant for hazelnut, peach, apple, kiwi, melon, tomato and carrot in adults, and for peanut, kiwi and banana in children (data not shown).

Although causal inference and the link with clinically manifest FA is limited in the data presented in ***Figure 3***, one interpretation of the observed inverse trends is that more frequent consumption may lead to less FS. Some support for this theory is found in studies on oral immunotherapy (OIT), which is associated with a reduction in levels of allergen-specific IgE.[109, 110] However, whether a reduction in level of specific IgE is a determinant of tolerance acquisition is unknown.[109, 110] Furthermore, it is notable that the foods showing a statistically significant inverse association in adults in our data are foods particularly known for involvement in PRFA. In ***Chapter 9***, we concluded that there is a potential beneficial effect of oral immunotherapy (OIT) with apple in pollen-related apple allergy, but the only study reporting laboratory endpoints found no significant change in IgE level to the Bet v 1 homologue in apple, Mal d 1, during OIT.[111] In fact, reverse causality as an explanation for the observation that only consumption frequency of pollen-related foods was significantly associated with sensitisation in adults is an important consideration. PRFAs generally present with mild OAS,[112, 113] and do not necessarily lead to complete avoidance of the culprit food. Adjusting the multivariable regression model for avoidance for these foods may not have been enough, because many of these sensitised subjects probably still consume the food, just less frequently or only in processed form, as heating diminishes the allergenic potential of pollen-related food allergens (***Chapter 9***).[112] Although PRFAs also occur in children, they are not quite as common as in adults, which could explain why the inverse relationship between consumption and sensitisation for pollen-associated foods was not as strong in children.

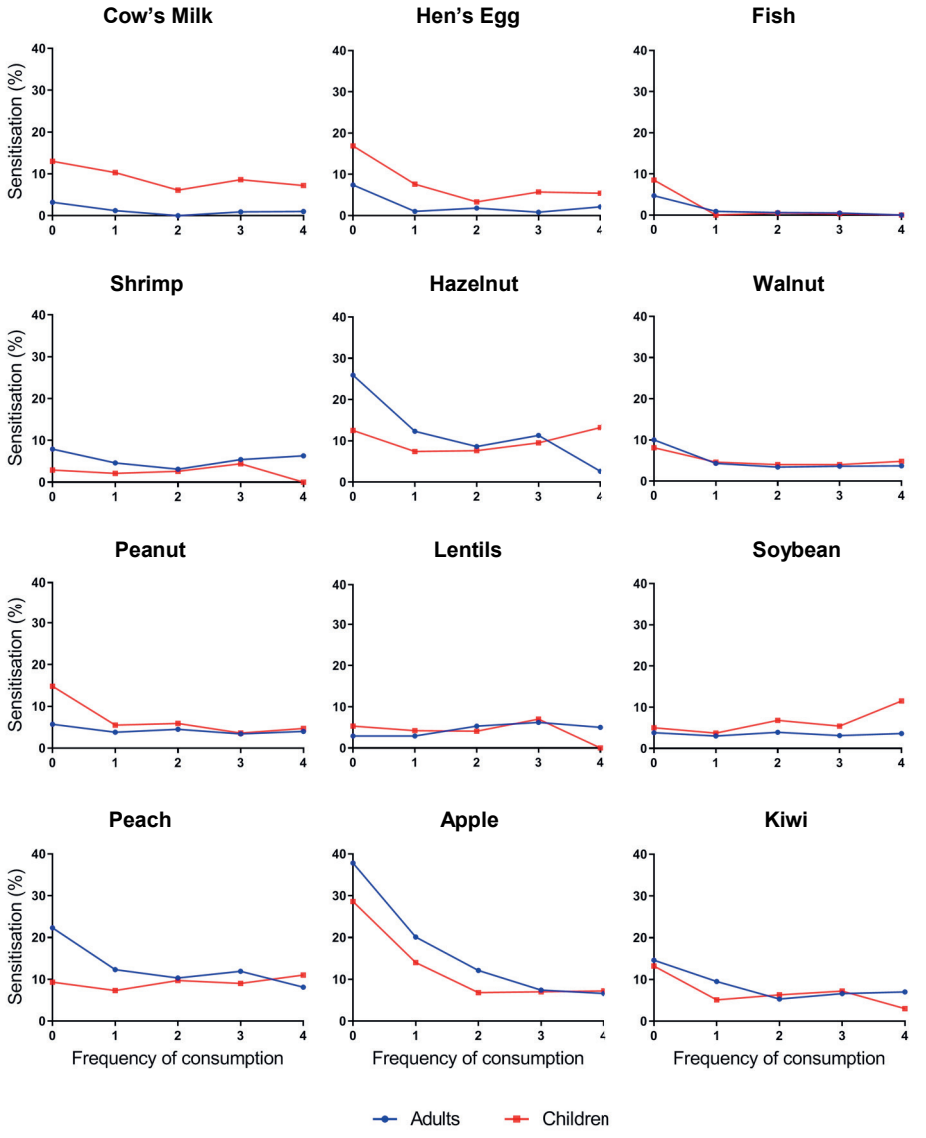

Figure 3. Relationship between frequency of consumption and sensitisation
Frequency of consumption: 0, Never; 1, <most months; 2, most months; 3, most weeks; 4, most days.

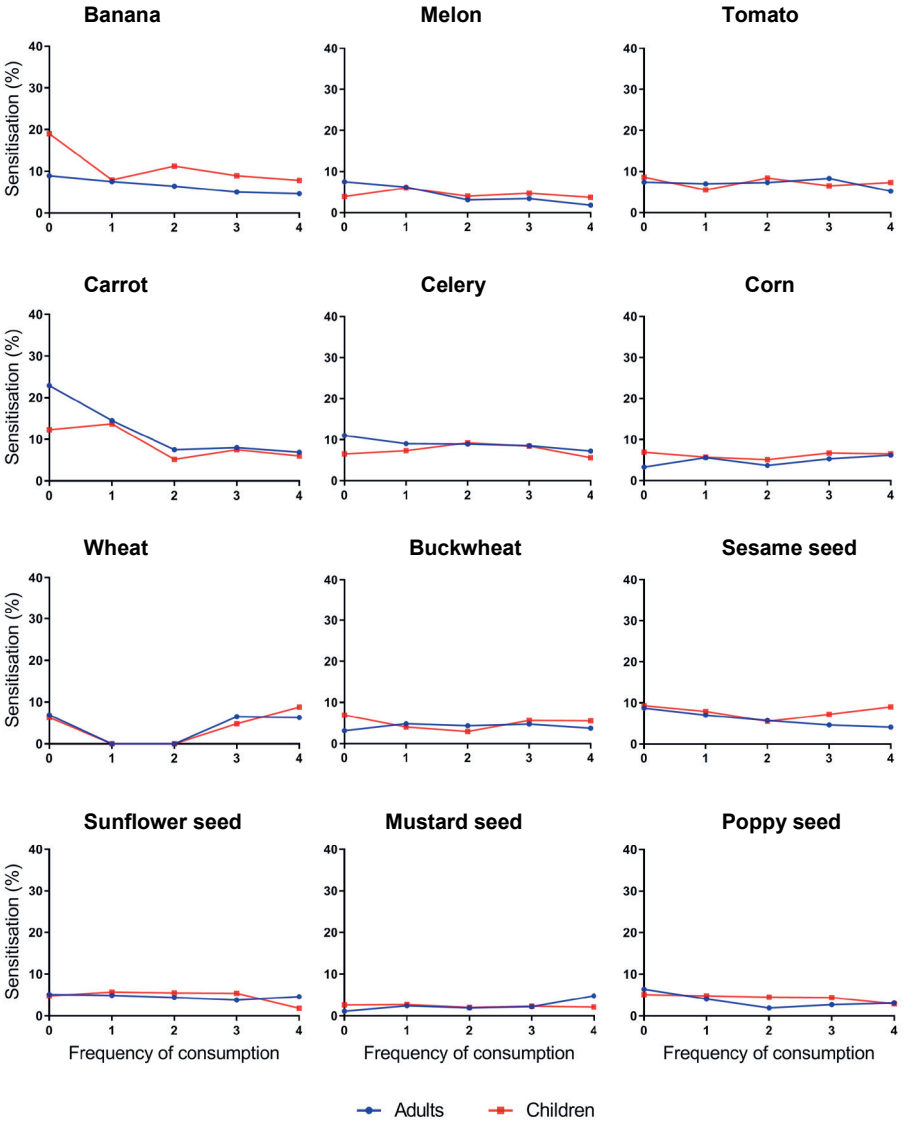

Figure 3. Relationship between frequency of consumption and sensitisation (continued)
Frequency of consumption: 0, Never; 1, <most months; 2, most months; 3, most weeks; 4, most days.

Previous studies on the association between frequency and/or quantity of consumption and FS or FA are scarce. Smits *et al*. found low correlation between peanut consumption in gram/day and prevalence of peanut sensitisation in the general population of all ages all across the world.[114] In the randomised, controlled Enquiring About Tolerance (EAT) trial, a statistically significant inverse association was observed between mean weekly consumption of peanut and hen's egg between 3 and 6 months of age, and probability of IgE sensitisation and allergy at 1 and 3 years of age in British children.[10] No such association could be determined for cow's milk, fish, sesame seed or wheat. Consumption of 2 grams per week of peanut or egg-white protein was linked to a significantly lower prevalence of these respective allergies than was less consumption.[10] Interestingly, this level of consumption corresponds with the median level of peanut consumption in Israeli infants, who have a much lower rate of peanut allergy than Jewish children in the UK. [10]

Our findings in combination with those of Smits *et al*. suggest that frequency and quantity of consumption at school-age or in adulthood plays, at most, a minor role in relation to FS in subjects from the general population. However, OIT studies have established that consistent exposure may be necessary to ensure sustained tolerance in sensitised subjects.[115] As such, the best recommendation for sensitised but tolerant subjects may be to keep consuming the food to which they are sensitised at regular intervals to prevent potential development of FA, as there is no evidence to the contrary. Furthermore, it is important to realise that higher quantities of certain foods (i.e. peanut and egg) consumed during infancy may prevent FA and help explain geographical differences.

6.1.2. Age of introduction

An alternative (non-mutually exclusive) theory is that early oral introduction to solid foods in infancy prevents FA. [9, 11, 116-119] In the randomised, controlled Learning Early About Peanut Allergy (LEAP) trial, 4- to 11-month-old infants with severe eczema, egg allergy, or both, were randomly assigned to either consumption of 6 grams of peanut protein per week until the age of 5 years, or avoidance of peanut until the age of 5 years.[9] Early consumption of peanut was found to reduce the development of peanut allergy by 80% by 5 years of age in these high-risk infants. The EAT trial confirmed the efficacy of early introduction of allergenic foods (between 3 and 6 months of age) for preventing FA in specific groups of high-risk infants at 1 and 3 years of age, though with respect to individual foods this was only statistically significant for hen's egg.[120] On the other hand, the EAT trial revealed no significant association between early introduction in normal (breast-fed) infants and risk of FA at 1 and 3 years of age.[10, 120] These observations correspond with our findings presented in ***Chapter 4*** and ***Figure 4*** that earlier introduction of solid foods in infancy showed a trend towards prevention of overall FS in school-age children from the general population, but that the association was not statistically significant.

Overall, systematic reviews from recent years conclude that early introduction of certain foods into the infant diet may be associated with a lower risk of developing FS and FA, specifically with regards to peanut and egg,[9, 11, 116-119] and especially in high risk children.[9, 120] The finding in the EAT study that the inverse association between early introduction and FA in normal breast-fed infants was statistically significant in the per-protocol analysis (as opposed to the intention-to-treat analysis) can be taken to mean that prevention of FA by means of early introduction of multiple allergenic foods also depends on adherence and dose.[10] All findings considered, the efficacy of early introduction of solid foods into the infant diet seems dependent on the risk profile of the child, the food concerned, and the frequency and amount of consumption.

Local dietary habits and regulations affect age of introduction to specific foods, which may be partly responsible for geographical differences in prevalence of FA. Until recently, many national guidelines advised avoidance of allergenic foods in infancy.[121, 122] Meanwhile, it is universally acknowledged that there is no evidence for delaying start of introduction of solid foods beyond 4-6 months in prevention of FA.[123] Updated European guidelines no longer impose dietary restrictions on pregnant or lactating mothers, and advise introduction of complementary foods to infant diet after the age of 4 months for all foods (including peanut and egg) and in all children (irrespective of atopic heredity), as long as the necessary neuromotor skills have developed.[124-126] Some guidelines suggest that complementary foods are ideally introduced before 6 months of age, though additional analysis of the LEAP data revealed that the probability of tolerance at 5 years of age was consistently higher with introduction of peanut between 6 and 11 months compared with introduction of peanut between 4 and 6 months.[127] The optimal time window for introduction of complementary solids remains to be consistently established.[125, 126]

6.1.3. Route of exposure

Paradoxically, several studies report that high household consumption of peanut gives a greater risk of peanut sensitisation, through increased levels of biologically active peanut allergen in household dust, particularly in children with atopic dermatitis (AD).[128-131] Induction of tolerance versus sensitisation and potential FA seems to depend not only on age (and possibly amount) of oral introduction, but also on route of antigen exposure.

Originally, both tolerance induction and primary FS were thought to occur exclusively via the gastro-intestinal tract.[23, 24] In 2008, the dual-allergen hypothesis was proposed.[23] According to this hypothesis, early high-dose oral exposure to food protein induces tolerance, and low-dose cutaneous exposure causes allergic sensitisation.[23, 24] Support for the theory has been found in several epidemiological and mechanistic studies. Infant AD predisposes to food sensitisation and allergy,

presumably as a result of skin barrier impairement.[132] Loss-of-function mutations in genes encoding the skin component filaggrin are associated with FS and allergy.[132-136] As regards the immunologic evidence, studies report that memory T cells expressing skin-homing receptor *cutaneous lymphocyte antigen* (CLA) proliferate more in response to peanut extract than memory T cells expressing gut-homing receptor α4β7,[137] and that CLA expression on peanut-specific effector T cells is increased in peanut allergic infants, whereas α4β7 expression is increased in non-allergic infants.[138] Cytokine responses trend towards T_H2 skewing (IL-4 and IL-13 production) in the CLA+ cells of peanut allergic children, and towards T_H1 skewing (IFN-γ and TNF-α production) in the α4β7+ cells of peanut tolerant children.[137, 139] However, two studies observed no differential expression of CLA or α4β7 on peanut-specific effector T cells of peanut allergic or tolerant children older than 1 year.[138, 140] Weissler *et al.* concluded that peanut allergy may only be associated with increased homing of peanut specific effector T cells to the skin in infancy.[138] Blom *et al.* suggest an alternative peanut-specific T_H2 cell phenotype in 1- to 5-year old children; an increased expression of skin (and airway) homing receptor CCR4 was observed in peanut allergic children compared to nonallergic children.[140] The latter findings support primary sensitisation to peanut via the skin or via the airways.[140] Although it is well established that pollen can lead to cross-reactive FS and PRFA, the role of respiratory sensitisation leading to primary FA remains to be further elucidated.[21] One study showed that mice exposed to α-lactalbumin via the gastro-intestinal, cutaneous or respiratory (intranasal) route were all sensitised, but sensitisation via the skin resulted in the highest IgE levels.[21, 141]

Geographical prevalence variations may also be related to the likelihood of cutaneous exposure to food allergens without concurrent oral introduction. For example, the high prevalence of sesame allergy in India may be caused by use of sesame oil as massage oil.[93] The high prevalence of peanut allergy in the UK, USA, Canada and Australia compared to Africa and Asia may be the result of comparable cutaneous exposure, but much later oral exposure in the Westernised countries due to original dietary guidelines for pregnant or lactating mothers or infants advising peanut avoidance.[23, 24, 121, 122] Perhaps similar reasoning underlies the high prevalence of seafood allergy in Spain, Greece and Iceland compared to the rest of Europe. Infants in these countries are likely to be environmentally exposed to fish and shrimp at a young age because of high consumption, but at the time of EuroPrevall data collection, guidelines still advised avoidance of oral introduction to fish and shrimp during infancy.[122]

However, if the dual-allergen exposure hypothesis holds true, one would expect FS and FA to decrease upon protection of the skin barrier. Contrary to these expectations, the majority of recent primary prevention trials using emollient therapy in infants with AD have shown no clear reduction in FS and FA.[142-145] Altogether,

development of FS and FA may be linked to, but does not appear to be limited to early transcutaneous exposure to the culprit food without concurrent oral exposure.

6.1.4. Processing

As mentioned in section 2 of this discussion, various methods of food processing may differently influence the allergenicity of foods.[39] Processing changes the structural and chemical properties of proteins, altering existing or creating new epitopes. This may affect the likelihood of inducing sensitisation (e.g. by reducing recognition by dendritic cells or influencing the mode of transepithelial transport), or the ability to elicit a reaction in allergic subjects (e.g. altering threshold doses or by impacting the capacity of allergens to cross-link receptor-bound IgE leading to degranulation).[39, 40] An illustrative example is that boiling (100 °C) reduces allergenicity of peanuts, whereas high-temperature roasting (120-280 °C) seems to enhance their IgE binding capacity.[39, 146] An explanation for the higher prevalence of peanut allergy in the USA than in China despite similar consumption rates, may therefore be that the Chinese generally boil or fry and Americans generally dry roast peanuts.[147] The majority of peanut on the European market is based on roasted products.[146] As of yet, it is unclear if and how differences in processing influence geographical variation in prevalence of peanut or other FS and FA on a European scale.[146]

6.1.5 Food exposure and geographical variation of FA prevalence

Considering all findings discussed in the previous sections, the prevailing opinion appears to be that FS and FA should be relatively low in regions where a food is consumed frequently, provided the food is introduced in sufficient amounts (at least 2 grams per week) at an early age (between 4 and 11 months). Why then, is relatively high prevalence of FA to certain foods observed in regions where these foods are consumed frequently? An important argument is that, until recently, FA guidelines recommended avoidance of allergenic foods in infancy. Most available European FA prevalence estimates, including the EuroPrevall findings, are based on data from subjects adhering to these guidelines. Subjects growing up in regions with frequent consumption of allergenic foods were more likely to be exposed to low (potentially sensitising) amounts of these foods during infancy, either via the GI tract or other routes (skin or respiratory), than subjects from countries with low levels of consumption of allergenic foods. It is conceivable that withdrawal of the avoidance recommendations from recent guidelines will reduce FA prevalence in regions with frequent consumption, making international FA prevalence estimates more comparable. Another essential realisation is that food allergic reactions can only occur when a subject is exposed to the culprit food in an allergenic form. Therefore, prevalence of FA based on accidental reactions is higher in regions where subjects are more likely to be exposed to the culprit food, especially to the culprit food in its most allergenic form (e.g. roasted peanut *vs* boiled peanut). Nonetheless, currently

available evidence cannot exclude the possibility that high consumption is associated with high risk of some food allergies, especially as the efficacy of early introduction has only been established for certain foods (peanut and possibly egg), and seems to depend on the patients' risk profile and consistent consumption.

Clinical implications

- No dietary avoidance should be imposed on infants, no matter the predetermined risk of allergy. Early oral introduction (between 4 and 6-11 months) of frequent sufficient amounts (at least 2 grams/week) of allergenic foods may prevent development FS and FA. Later oral introduction may increase likelihood of FS and FA by allowing more time for exposure to lower (potentially sensitising) amounts of allergenic food, either via the GI tract, the skin or the respiratory route.
- Health professionals may want to advise tolerant but sensitised subjects to maintain regular consumption of the concerned food where possible, as the EuroPrevall data revealed that increased frequency of consumption is not linked to increased likelihood of FS, and studies on OIT show that avoidance of the culprit food after completion of therapy is associated with recurrence of symptoms.

6.2. Pollen exposure

A considerable proportion of all plant FAs are caused by cross-reacting allergenic structures shared by pollen and foods.[35, 36, 112, 113] As vegetation is region-specific, so is the likelihood of certain pollen-food cross-reactivity. Some of the main allergens involved in clinically relevant pollen-food cross-reactivity are pathogenesis-related protein family (PR-10) proteins, non-specific lipid transfer proteins (LTP), and profilins.[35, 36, 112, 113, 148]

The primary sensitising agent for PR-10 proteins is major birch pollen allergen Bet v 1. Bet v 1 is renowned for cross-reacting with similar proteins in tree nuts, *Rosaceae* fruits, and *Apiaceae* vegetables, and leading to generally (but not exclusively) mild oral allergy symptoms. [112, 113, 149] As discussed in **Chapter 2 and 3** and shown in **Figure 2**, PR-10 sensitisation is common amongst plant food sensitised Europeans, mainly due to frequent occurrence in the Netherlands, Switzerland, Poland and Lithuania, where birch-pollen is endemic.[150] Birch PRFA at least partly explains the overall high prevalence of probable FA and the dominance of hazelnut, apple, peach, kiwi, carrot and celery allergy in Northern and Central Europe.

By contrast, allergy to *Rosaceae* fruits and tree nuts in the Mediterranean area is partly related to LTP-sensitisation.[35, 36, 151, 152] Peach LTP Pru p 3 is generally considered the primary sensitiser. [151, 152] The route of primary sensitisation is unclear - most likely via the gastro-intestinal system or across the skin.[151, 152] These reports suggest LTP-associated FA is food- rather than pollen-induced. However, it is unclear

why LTP-associated FA is largely confined to the Mediterranean area,[35, 36, 151, 152] when LTP-containing plant foods are consumed throughout Europe (albeit to different extent). Pollen may partly explain this geographical distribution. LTPs constitute important pollen allergens of species more native to Southern than Northern Europe, such as mugwort, plane tree, olive tree, pellitory, and cypress.[150-152] Mugwort and plane tree have been described to have the ability to act as primary LTP sensitisers.[153-156] Alternatively, pollen allergens may function as co-factors. For example, the risk of sensitisation to apple LTP appears to be decreased in patients suffering from birch pollinosis, but increased in patients with mugwort or plane pollen allergy.[157] Recognition of LTP is often, but not exclusively, associated with severe symptoms.[35, 152]

Profilins are true pan-allergens to which primary sensitisation seems to arise via (often grass) pollen, and which can cross-react with homologues from virtually every plant source (food), in any part of Europe.[35, 158, 159] Sensitisation to food profilins is common, but their clinical relevance in FA is an ongoing matter of debate.[158] They have been associated with mostly mild clinical reactions to Rosaceae fruits, tree nuts, melon, tomato, and banana in Southern Europe.[158, 160, 161] Potential roles for profilin have also been suggested in birch-celery syndrome (besides PR-10) in Northern and Central Europe, mugwort-celery syndrome in Central Europe, and mugwort-peach and plane-fruit syndrome (besides LTP) in Southern Europe.[159] Profilin-related FA may be relatively more relevant outside birch territory.

Accordingly, a particularly illustrative example of typical plant source FS patterns across Europe was observed for walnut in **Chapter 7**: sensitisation to Jug r 5 (PR-10 protein) in Northern and Central Europe; sensitisation to Jug r 3 (LTP) in the Mediterranean; and sensitisation to profilin (Jug r 7) throughout Europe. Both Jug r 5 and Jug r 7 sensitisation were found to predictive of mild-to-moderate rather than severe walnut allergy, though our data did not confirm a significant association between Jug r 3 sensitisation and walnut allergy phenotype.

It is worth briefly returning to the topic of heat processing, as this is a particularly important consideration in the context of PRFA. PR-10 proteins and profilins denature upon heating, whereas LTPs and storage protein are mostly heat stable.[35, 36, 40, 112, 113] Therefore, hazelnut and apple allergic individuals in Northern and Central Europe are on average less likely to react to heat-processed forms of the food than those in the Mediterranean. Furthermore, as discussed in **Chapter 9**, the effect of heating does not apply equally to all foods implicated in PRFA.[40, 152, 162] Bet v 1 proteins in apple (Mal d 1), carrot (Dau c 1), celery (Api g 1) and hazelnut (Cor a 1) begin to change structure at respectively 28 °C, 43 °C, 50 °C and 100 °C.[162] Api g 1 returns to its native structure after cooling, where Mal d 1 and Dau c 1 do not.[162]

Clinical implications

- In patients presenting in Northern and Central Europe with allergies to *Rosaceae* fruits or tree nuts, PRFA caused by PR-10 cross-reactivity should be considered, whereas in Southern Europe, LTP-related FA is more relevant. This is also important in relation to severity of symptoms, as the former generally presents with mild symptoms, and the latter relatively more often with severe symptoms.

6.3 Microbial exposure

In ***Chapter 4***, we observed that dog ownership in early childhood was inversely associated with FS in later childhood, an association that was also detected in previous studies.[163-165] Our study did not confirm the relevance of other postulated early-life environmental risk factors for FA after mutual adjustment for other environmental exposures, infant diet and demographics. However, univariable analysis did suggest preventative tendencies ($p<0.2$) of having multiple (older) siblings, day care attendance, bedroom sharing, or growing up in a farm environment (***Figure 4***).

It is conceivable that these environmental determinants are markers for increased or more diverse microbial exposure in infancy. The microbiome is thought to play an important role in determining the likelihood of developing FS and FA, as described in the context of the biodiversity hypothesis.[17, 19, 104, 105] An American study in human faecal samples found that adults with peanut and tree nut allergies had lower gut microbiota diversity compared to non-allergic subjects.[166] A murine study showed that transfer of the gut microbiota from FA-prone to germ-free mice passed on the FA phenotype.[167] In another murine study, researchers found that administration of certain bacterial strains led to induction of regulatory T cells and reduced FS.[168] The particular association between dog ownership and reduced FS may be caused by more diverse household microbiota, or perhaps by more out-door activity, compared to non-dog owners.[165] Diversity and evenness of the bacterial microbiome in household dust was found to be associated with dog ownership (and not with cat ownership).[169]

It is of interest that the relevance of timing of oral introduction to allergenic foods can be explained through the biodiversity hypothesis as well as through the dual-allergen hypothesis, if early introduction leads to more robust development of the gut microbiota.[170] A prospective birth cohort study of children from rural areas in European countries found that increased diversity of food within the first year of life was associated with increased expression of a marker for regulatory T cells, and inversely associated with FS and FA at 6 years of age.[171]

On a European scale, microbial exposure does not evidently clarify differences in FA prevalence between the EuroPrevall centres. However, it should be acknowledged that the EuroPrevall participants were recruited from cities rather than from rural areas. In ***Chapter 4***, we saw that 5% of adults and 0.5% of children included in the study grew up in a farm environment. In adults, growing up on a farm was inversely (but not statistically significantly) associated with FS (***Figure 4***). In children, too few study subjects (N=10) were exposed to a farm environment at a young age to validly evaluate the association with FS. If living on farms or in more rural areas leads to a more diverse microbiome than living in cities (e.g. through more exposure to plants, animals or soil), and if a more diverse microbiome does indeed protect against development of FA, then European FA prevalence estimates may be lower if these rural areas are taken into account, especially in the least urbanised countries.[78, 104, 172-174]

On a global scale, more (diverse) microbial exposure in developing than in Westernised parts of the world may partly explain their lower prevalence of FA compared with Europe, the USA and Australia. Parasitic infections may also play a key role. Parasites thrive in the tropical climates of most underdeveloped countries.[175-177] Despite the fact that both allergies and parasitic infections are characterised by a strong T_H2 immune response and elevated levels of IgE, studies have found that these diseases are inversely related to one another.[175, 178, 179] The immunological basis for this inverse association is unclear, but parasites are known to employ immunomodulatory mechanisms to prevent an inflammatory response and their subsequent elimination from the human body.[175, 177, 179-181] A side-effect of these anti-inflammatory signals may be the suppression of an allergic reaction, despite presence of allergen-specific IgE. For example, studies have shown that helminth infections can direct proliferation of regulatory T cells, which can induce hyporesponsiveness to antigens.[175, 177, 179, 180] Alternatively, very high levels of parasite-induced IgE and IgG may block the binding of allergen-specific antibodies to effector cells.[180, 181] Furthermore, the immunosuppressive capacity of parasites has been linked to their ability to alter the composition of the host microflora.[179, 180, 182] Theoretically, any of the aforementioned effects could help explain the low prevalence of probable FA observed in the INCO study in India, despite the high prevalence of FS. Children in helminth-infected populations have previously been demonstrated to have high levels of allergen-specific IgE in the absence of symptomatic allergic disease.[175, 181, 183] In reality, the observations in the INCO study that parasitic infections were detected in less than 20% of the population and that occurrence was similar in subjects with and without symptoms to foods, contradict this idea, and additional explanations for the high rates of FS in Asian countries are needed.[176] Perhaps, rather than parasitic infection preventing a food allergic reaction, tropomyosins or CCDs in parasites and mites lead to cross-reactive FS,[175, 178] with

cross-reactivity based on tropomyosins potentially explaining the high levels of clinically irrelevant shrimp sensitisation in India.[93]

Clinical implications

- Notwithstanding other valid reasons, fear to develop FA should not prevent parents from getting a dog, as dog ownership in infancy is associated with reduced prevalence of FA.
- Parasites may induce (cross-reactive) FS, but may also prevent clinical manifestation of FA.

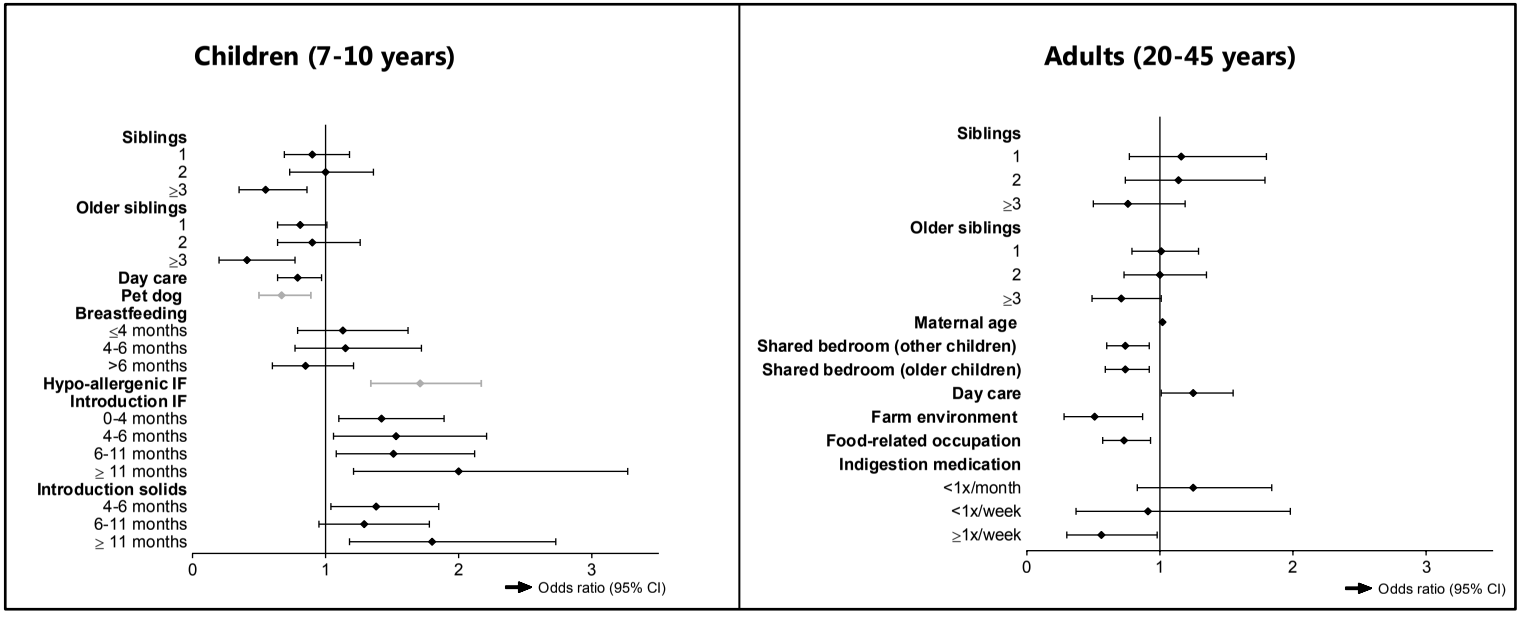

Figure 4. Environmental predictors of food sensitisation; univariable analyses; p<0.2
Green represents the environmental predictors that remained significant in multivariable analyses. **Environmental determinants not shown (p>0.2) for food sensitisation in childhood:** maternal age, shared bedroom with other or older children, farm environment, inner city environment, pet cat, serious respiratory infection, antibiotics, maternal smoking during pregnancy, maternal or paternal smoking during childhood, reflux medication in last 6 months, vitamin D supplementation, cow's milk IF, soy milk IF. **Environmental determinants not shown (p>0.2) for food sensitisation in adulthood:** inner city environment, pet dog, pet cat, serious respiratory infection, smoking. Early-life exposures for childhood FS: <2 years; for adulthood FS: <5 years. *IF, infant formula.*

II. FOOD ALLERGY IN THE PRESENTING PATIENT

The EuroPrevall project also extended to outpatient populations, i.e. to "the presenting patient" in clinical practice. Where questions of etiology and prevention command the majority of research interest in the general population, topics related to diagnosis, prognosis and treatment become more relevant in the presenting patient. ***Chapter 5 to 8*** of this thesis focused on gaining insight into the clinical profile of patients with FA and evaluating the predictive value of individual and combined diagnostic modalities. As PRFA (increasingly) dominates the image of FA in Europe, ***Chapter 9*** was devoted to dietary management and interventions in these particular patients.

Part II of this general discussion will explore the main findings in ***Chapter 5 to 9*** of this thesis in the following sections:

1. Can patient history accurately predict FA?
2. Can clinical background determinants accurately predict severity of FA?
3. Can IgE testing improve prediction of FA in addition to patient history?
4. Can't we just use CRD to predict FA instead?
5. The unjustified underdog status of pollen-related FA

1. Can patient history accurately predict FA?

Patient history is considered a key tool in diagnosis of FA. Clinical experience has taught FA specialists the main determinants of an IgE-mediated reaction. Based on these expert opinions, current guidelines state that timing, reproducibility, type of symptoms and co-existing allergic diseases should be addressed in patient history for FA.[184-186] These same guidelines conclude that expert opinion is not enough, and emphasise the need for studies investigating the value of patient history using standardised allergy-focused questionnaires.[184] In ***Chapter 5***, we addressed this major gap in the evidence of FA diagnostic workup; scientifically reinforce the value of (specific features of) patient history; and provide direction and focus topics for GPs and other non-allergy specialists taking patient history for FA, which are in accordance with current guidelines.

Figure 5 displays determinants from patient history in adults and school-age children that contribute to accurate prediction of IgE sensitisation in children and adults with symptoms to food (i.e. probable FA). As discussed in ***Chapter 2 and 3***, probable FA to animal source foods is relatively common in children, whereas probable FA to plant source foods dominates in European adults. In ***Chapter 5***, we observed that prediction of probable FA using patient history was more accurate in adults (AUC 0.85) than in children (AUC 0.73), but that accuracy in children improved when focusing on plant source foods (AUC 0.81). Reporting of OAS or of AR

comorbidity was found to be strongly predictive of probable FA in both children and adults. Although OAS are typically associated with PRFA, they are generally the first symptoms in any IgE-mediated reaction to food.[48, 187] In accordance, OAS remained an independent predictor of probable FA after adjustment for Bet v 1 sensitisation in both children and adults in our study.

Observed differences between children and adults in our study also have implications for interpretation of patient history in clinical practice. Reporting of gastrointestinal (GI) symptoms (vomiting or diarrhoea) was associated with absence of probable FA in adults, but only with absence of probable FA in children when focusing on plant source foods. Apparently, GI symptoms are rarely reported in association with plant FA. In contrast, over 50% of children with animal source probable FAs reported GI symptoms. Besides the type of causative foods, parents' observations and actions also influence the value of patient history in children.[188] The finding that time until reaction onset and reproducibility of the reaction were not independent predictors in school-age children may respectively be explained by parents not realising their child is having an allergic reaction until objective symptoms appear, and parents ensuring strict avoidance of a one-time offending food.

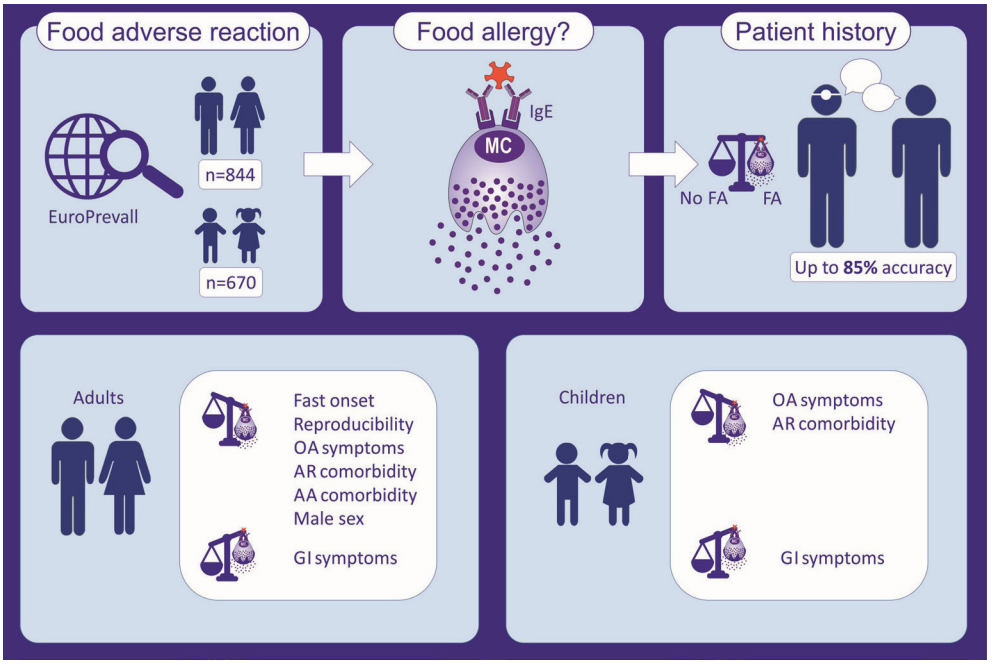

Figure 5. Patient history determinants predictive of FA
OA, oral allergy; AR, allergic rhinitis; AA, allergic asthma; GI, gastrointestinal

Our findings serve first and foremost to scientifically substantiate current guidelines on patient history for FA.[124] Overall, patient history can distinguish between presence and absence of probable FA, but the predictive value of patient history depends on the patient population (adults *vs* children), on the type of offending food (plant *vs* animal source), and probably on geographical location (birch-endemic areas *vs* outside birch territory).

Of future interest for clinical practice is the finding that a multivariable model including all independent predictors for probable FA in adults had a high negative predictive value (95% at a prediction score cut-off of 0.17). Using this cut-off, almost half the adults reporting food-related symptoms could be classified as not having probable FA, with only a 5% chance of a false-negative prediction. After external validation of our findings and subsequent development of a practical application, our prediction rule could function as a complementary decision tool for GPs and non-allergy specialists, to provide extra certainty regarding absence of FA, and prevent unnecessary IgE testing or referral.

Clinical implications

- Patient history for FA should address the time until onset of reaction, reproducibility of the reaction, symptoms (particularly OAS and GI symptoms), and allergic comorbidities (including allergic asthma and allergic rhinitis).
- Patient history incorporating above reaction characteristics, allergic comorbidities and demographics, can be used to rule out probable FA, and may prevent unnecessary IgE testing, particularly in adults presenting with adverse reactions to plant source foods.

2. Can clinical background determinants accurately predict severity of FA?

Patients and their health care providers are generally not only interested in whether or not they have a FA, but also in the severity of their FA. ***Table 1*** presents clinical background variables independently associated with mild-to-moderate or severe allergic reactions to hazelnut,[189] walnut (***Chapter 7***) and peanut (***Chapter 8***) in the EuroPrevall outpatient population of children and adults.

Some expected and some intriguing parallels between the profiles of patients with mild-to-moderate and severe allergies to tree nuts and peanut are observed. Unsurprisingly, (birch) pollen allergy was associated with a mild-to-moderate phenotype of tree nut and peanut allergy in this population of subjects mostly from birch-endemic regions.[112, 113, 149, 190] Although birch pollen allergy was not selected as an independent predictor of mild-to-moderate walnut allergy, the direction of the association was similar to those of peanut and hazelnut allergy. PRFA, especially birch PRFA, is considered a benign condition in which systemic reactions are rare, though

exceptional severe reactions have been reported.[48, 190, 191] It is unknown why some patients experience more severe symptoms; whether this is related to specific foods, seasonal 'priming' of the airways, co-sensitisation to more stable allergens such as seed storage proteins or LTP, or diagnostic misclassification.[190, 192] The association of mugwort pollen allergy with severe walnut allergy could theoretically be caused by overlapping sensitisation to LTPs in mugwort and walnut.[154-156] Alternatively, still uncharacterised mugwort allergens may be responsible for the severe walnut allergic reactions described in relation to mugwort PRFA.[112]

Table 1. Clinical background characteristics for prediction of severity of tree nut and peanut allergy

Reaction	Hazelnut*	Walnut	Peanut
Mild-	Pollen allergy	-	Birch pollen allergy
Moderate	-	Cat/dog sensitisation	-
Severe	Atopic dermatitis	Atopic dermatitis	Atopic dermatitis
	Latex allergy**	-	Latex allergy
	-	Symptoms on skin contact	Symptoms on skin contact
	-	Family atopy	Family atopy
	-	Mugwort pollen allergy	-
	-	-	House dust mite allergy
	-	-	Age at onset < 14 years
	-	-	Female sex

*Data on predictors for severity of hazelnut allergy (HA) were extracted from Datema *et al*.[189] The presented predictors for severity of HA were determined in subjects with self-reported HA and in subjects with challenge-confirmed HA, whereas the predictors for severity of walnut and peanut allergy were determined in subjects with probable walnut and peanut allergy. **Latex allergy was no longer a statistically significant predictor in the subpopulation of subjects with challenge-confirmed HA.

Interestingly, some of the strongest independent predictors from clinical background associated with severe hazelnut, walnut and peanut allergy appear to be related to skin reactivity: ever having had atopic dermatitis (hazelnut, walnut, peanut), having symptoms elicited by skin contact with the culprit food (walnut, peanut), and having a latex allergy (hazelnut, peanut). In ***Chapter 7 and 8***, we hypothesised that cutaneous route of sensitisation may not only be associated with development of FA as proposed in the dual allergen hypothesis,[23, 24] but also with a more severe phenotype of FA. Further speculation led to the realisation that sensitisation via the skin probably leads to primary (non-cross-reactive) sensitisation *relatively* more often than sensitisation via the respiratory tract, as pollen induces the most common type of cross-reactive FS (presumably) via the respiratory tract. In other words, skin-related variables may be predictive of severe FA, because they are indirect markers of FA other than (mostly mild) PRFA.

A family history of atopic disease was also predictive of severe reactions to walnut and peanut. The effect direction was similar, but not statistically significant, for hazelnut.[189] A previous study found no association between family history of atopic

disease and the severity of an accidental or DBPCFC-provoked reaction to food in a paediatric population.[193] Regarding specific foods, the same study observed that paternal asthma was predictive of severe hazelnut allergy but of mild cashew nut allergy, that paternal atopic dermatitis was associated with mild hazelnut allergy, and that maternal asthma was associated with mild peanut allergy.[193] These contradictory findings suggest that the association between family atopy and FA phenotype differs depending on the type of atopic disease and the type of food. In reality, it is likely that the association between family atopy and FA phenotype is subject to a complex interplay between (shared) genetics, environmental exposures and/or behavioural traits.[194] More research is needed to truly understand how family atopy is associated with FA phenotype.

Table 2. Area under the curve estimates for prediction of severity of tree nut and peanut allergy

	Hazelnut	**Walnut**	**Peanut**
Outcome	*Self-reported/ DBCPFC-confirmed FA*	*Probable FA*	*Probable PA*
CB	0.62 / 0.75	0.74	0.74
SPT extract	0.57 / 0.72	0.54	0.63
IgE extract	0.54 / 0.61	0.54	0.63
CRD	0.66 / 0.76	0.66	0.65
CB + extract	NA	0.74	0.74
CB + extract + CRD	0.70 / 0.86	0.81	0.75

See Datema *et al*,[189] Chapter 7 and Chapter 8 for 95% confidence intervals See Table 1 for variables in each CB model. The CRD model included level of IgE to Cor a 1 (mild) and Cor a 9 (severe) for hazelnut; Jug r 1 (mild), Jug r 2 (severe), Jug r 4 (mild), Jug r 5 (mild), Jug r 7 (mild) and Ana c 2 (mild) for walnut; Ara h 2/6 (severe) and Ara h 8 (mild) for peanut. In addition to the CB model variables, the CB/extract model contained positive SPT for walnut (severe), and SPT ratio (severe) and IgE level (severe) for peanut. In addition the CB/extract model variables, the CB/extract/CRD model contained IgE level to Cor a 14 (severe) for hazelnut; Jug r 1 (mild), Jug r 5 (mild), Jug r 7 (mild) and Ana c 2 (mild) for walnut; and Ara h 1 (severe), Ara h 2/6 (severe) and Ara h 8 (mild) for peanut. *CB, clinical background; CRD, component-resolved diagnostics; SPT, skin prick test; NA, not available.*

As viewed in ***Table 2***, correct distinction between mild-to-moderate and severe allergy was approximately 75% likely for all three foods using just the clinical background determinants presented in ***Table 1*** (and the DBPCFC outcome rather than self-reported outcome for hazelnut allergy).[189] Some previous efforts have been made to examine possible predictors of severe food allergic reactions,[48, 50, 193, 195-202] but prior research evaluating to what extent the severity of food allergic reactions can be predicted by combined information directly available from clinical history is lacking. One Dutch study by Pettersson *et al.* concluded that severity of FA is largely unpredictable based on a multivariable model combining clinical background predictors with measurements of IgE sensitisation and eliciting dose during DBPCFC.[193] In the awareness that the AUCs of 0.75 in our studies are only moderately accurate, and that the presented predictor combinations may mainly distinguish between PRFA and primary FA, our findings still contrast with Pettersson *et al.*'s conclusion. Differences in study methodology limit extensive comparison between

the study by Petterson *et al.* and our findings, but it should be remarked that the choice of study population (entirely paediatric *vs* mostly adult) may play a role in the contradictory conclusions.

Our findings suggest that the right combination of clinical background determinants *can* predict severity of accidental reactions to some degree. Based on the deduction that many of the predictors in our models seem to be related to distinction between (mild) PRFA and other FAs, it is conceivable that our models may only be useful with regard to plant source foods, particularly in the birch-endemic regions of Europe, and more so in adults than in children. The clinical applicability of such models remains to be determined. Although there is some overlap between the selected predictors in the models for hazelnut, walnut and peanut, models to optimise prediction in clinical practice should probably be food-specific. To gain more insight, our findings should be externally validated for tree nuts and peanut, but also for other foods. The severity outcomes should be based on symptoms experienced during accidental reactions as well as during DBPCFC, as low correlation has been found between severity of reported reactions in the community and severity of reactions elicited during DBPCFC.[203]

Other potentially relevant determinants available from patient history include the symptoms/severity of previous reactions, the amount of an allergen consumed to elicit a previous reaction, frequency of past reactions (or reproducibility), time until onset of symptoms, reactions to raw *vs* processed food, co-existing food allergies, reason for avoidance of the culprit food (previous reaction, IgE sensitisation determined in the past, aversion, co-existing allergy to another food), and augmenting factors such as alcohol, exercise, or medication.[48, 50, 193, 195-202, 204] These determinants should be considered alongside our findings in table 1 in future evaluation of prediction of severity based on patient history.

Clinical implications

- Information available from patient history on clinical background (demographics, allergic predisposition) contributes to prediction of FA severity, but further research is required to strengthen and confirm clinical applicability.

3. Can IgE testing improve prediction of FA in addition to patient history?
After patient history, important tests in the diagnostic workup of FA include skin prick testing (SPT) with food extract, measurement of serum IgE to food extract, and measurement of serum IgE to food allergen components (component-resolved diagnostics, CRD).[184, 205] Guidelines state that specific allergy testing should be directed by patient history, to avoid identifying clinically irrelevant FS.[184, 206] Despite this recommendation, few studies have examined the predictive value of IgE sensitisation tests in addition to clinical background determinants available from patient history. Such combinations, if diagnostically accurate, have the potential to reduce the number of resource-intensive and burdensome DBPCFCs. For example, investigators in the UK and Ireland found that food challenge outcome in children could be accurately estimated using a prediction rule combining age, sex, symptom severity according to patient history, SPT wheal size, level of IgE to extract, and total IgE minus level of IgE to extract. The corresponding AUCs for peanut, egg and milk were 0.97, 0.95 and 0.94 respectively.[207] The high discriminative ability of aforementioned prediction rule for predicting peanut challenge outcome was confirmed in Dutch children (AUC 0.88), though calibration was poor.[208] In an update of the model, peanut CRD results (Ara h 1, 2, 3 and 8) were offered as additional candidate predictors, but did not replace the clinical predictors and did not improve prediction. A model combining sex, SPT wheal size, specific IgE to peanut extract and total IgE minus specific IgE to peanut extract had the highest diagnostic accuracy (AUC 0.94). However, IgE to Ara h 2 alone performed just as well as the multivariable model (AUC 0.90).[208] In adults, IgE to Ara h 2 (AUC 0.76) even performed better than the multivariable model (sex, SPT wheal size, specific IgE to peanut extract and total IgE minus specific IgE to peanut extract; AUC 0.64), though both the multivariable prediction model and Ara h 2 had lower diagnostic accuracy in Dutch adults compared to children.[209, 210]

Similar attempts at combining clinical background with measures of IgE sensitisation for estimating the risk of a severe reactions are even more scarce. Pettersson *et al*. found that SPT ratio and level of IgE to extract, in addition to age, eliciting dose, reaction time, and symptom severity, were independent predictors for the severity of a DBPCFC reaction to milk, egg, peanut, cashew nut and/or hazelnut in children, but that these variables explained less than a quarter of the variance in severity.[193] The additional value of CRD was not investigated. ***Table 2*** gives an overview of the individual and combined accuracy of clinical background, extract-based tests, and CRD for predicting reaction severity in hazelnut allergy,[189] walnut allergy **(Chapter 7)** and peanut allergy **(Chapter 8)** in the EuroPrevall outpatient population of children and adults. A model combining all components of FA diagnostic workup achieved best prediction of severity for each food, but improvement of prediction compared to clinical background alone was only statistically significant in hazelnut and walnut allergy, and not in peanut allergy. The AUCs in ***Table 2*** suggest that improvement of

the AUC was not caused by addition of the extract-based test results, but by the CRD results. It was also notable that, with the exception of cat/dog and Jug r 1 sensitisation for walnut allergy, all variables predictive of a mild-to-moderate phenotype of tree nut or peanut allergy were indicative of cross-reactive FS: (birch) pollen allergy, IgE to Cor a 1, Jug r 5, Jug r 7, Ara h 8, and CCD (see legend Table 2). A probable reason that CRD testing did not improve prediction of severity of peanut allergy in contrast to the models for tree nut allergy, is that a smaller proportion of peanut allergies is due to pollen cross-reactivity. Taxonomically, tree nuts are much more closely related to tree pollen than peanuts and other legumes. Cor a 1 has 67% amino acid sequence identity with Bet v 1,[211] whereas for Ara h 8 this is 46%.[212] Higher sequence homology makes clinically relevant cross-reactivity more likely, possibly by leading to higher antibody binding affinity.[213]

Clinical implications

- Prediction of severity of hazelnut and walnut allergy improves by addition of extract-based test results and CRD results to clinical background determinants.
- Such multivariable prediction models not only provide insight into the clinical profile of patients with mild-to-moderate and severe tree nut allergy, but have the future potential to support clinical decision-making in patients with unknown reaction severity, and maybe even reduce the number of DBPCFCs (after further development and validation).

4. Can't we just use CRD to predict FA instead?

Of course, it would be more practical if a single standardised test rather than a multivariable model could be used to accurately predict FA and severity of FA, like Ara h 2 and Ara h 6 can predict presence of peanut allergy.[208-210, 214] However, in ***Chapter 6*** we observed that hazelnut extract and CRD could not distinguish presence from absence of challenge-confirmed hazelnut allergy in a population of Dutch adults either, whether or not hazelnut allergy was limited to objective symptomatology. Furthermore, although CRD contributed to more accurate prediction of severity of hazelnut and walnut allergy, ***Table 2*** reveals that CRD alone had poor discriminative ability regarding severity of hazelnut, walnut or peanut allergy in the EuroPrevall mixed paediatric and adult population.

Comparison of our findings to literature confirms that measures of diagnostic accuracy depend on multiple factors, including the population (adults *vs* children), the investigated food (tree nuts *vs* peanut) and the outcome (FA *vs* severity of FA). IgE to hazelnut storage components Cor a 9 and Cor a 14 had poor diagnostic accuracy for hazelnut allergy in adults in ***Chapter 6*** (AUC 0.57 and 0.62 respectively) and in another previous study in Dutch adults (AUC 0.66 and 0.67);[215] slightly better diagnostic accuracy in a mixed population of children and adults (AUC 0.70 and

0.71);[189] and high diagnostic accuracy in entirely paediatric populations (AUC up to 0.80 and 0.89).[216, 217] Where prediction of hazelnut allergy using CRD in adults appears to be inaccurate, studies have shown considerably higher AUC estimates for prediction of peanut allergy using CRD in adults (AUC 0.76-0.85)[209, 210] and also children [AUC 0.90-0.99][208, 218-221]. However, where CRD can accurately estimate the risk of peanut allergy in adults, the accuracy of CRD for estimating risk of severe peanut allergy in adults is poor, as shown in ***Chapter 8*** (AUC 0.65) and in a previous Dutch study (AUC 0.58-0.65).[209]

Again, the high prevalence of cross-reactive FS through (birch) pollen in Europe, alongside the theory that sensitisation to Bet v 1 homologues is more clinically relevant in tree nut than peanut allergy, appears to play an important role in the described age- and food-related differences. As discussed in ***Chapter 6***, the vast majority of Dutch adults with hazelnut allergy are sensitised to hazelnut Bet v 1 homologue Cor a 1, whereas most hazelnut allergic children are sensitised to hazelnut storage proteins Cor a 9 or Cor a 14, but not to Cor a 1.[222] Because symptoms of birch PRFA are subjective, generally mild, and dependent on (heat) processing and season, it is not surprising that IgE to Cor a 1 is poorly associated with hazelnut challenge outcome. [30, 112, 223] As most Dutch hazelnut allergic subjects have isolated Cor a 1 sensitisation, this leads to overall lower sensitivity (and inherently AUC) of Cor a 9 and 14. Sensitisation, especially mono-sensitisation, to Cor a 9 and/or 14 is uncommon in Dutch hazelnut allergic adults. This made it difficult to examine the true association between hazelnut storage protein sensitisation and hazelnut allergy. One may conclude, however, that the true association is of lesser clinical relevance because of low prevalence in this population. Our results raise the question whether testing for IgE sensitisation to hazelnut extract and components in adults presenting with symptoms to hazelnut in birch-endemic regions should remain standard practice, or whether we should move straight to food challenge in this population. If so, DBPCFC should be the standard, because of the subjective nature of birch pollen-related hazelnut allergy.

Clinical implications

- CRD (specifically testing for IgE to 2S albumins) can accurately predict peanut and hazelnut allergy in children, and peanut allergy in adults, reducing the need for DBPCFC in these populations.
- CRD does not predict hazelnut allergy in adults from birch-endemic regions. For these patients, DBPFC remains the gold standard test.
- CRD does not accurately predict *severity* of hazelnut, peanut or walnut allergy in children or adults. DBPCFC remains the test of choice to gain insight into severity, but multivariable models combining CRD results with clinical background determinants may complement diagnosis in the future.

5. The unjustified underdog status of pollen-related FA

Some investigators remark on the inclusion of patients with PRFA in FA research as a study limitation leading to overestimation of FA prevalence, and advocate the exclusion of such subjects.[224] However, in both adult and older paediatric populations from birch-endemic countries like the Netherlands and Switzerland, this makes no sense. It would create a clinically irrelevant study population, as the majority of presenting patients have birch-PRFAs. The importance of determinants associated with cross-reactive FS in prediction of FA in ***Chapter 5 to 8*** of this thesis corroborate the common occurrence and clinical relevance of PRFA. As prevalence of pollen allergy is reported to be increasing, so will the prevalence of PRFA.[112, 113] Rather than denying its existence, we should focus on competent management.

The cornerstone for treatment of FA is accurate dietary advice.[184] As with all FAs, dietary avoidance advice for PRFA should be patient tailored, and depend on the severity of their symptoms and the eliciting dose.[184] However, the fact that labile proteins are responsible for PRFA, means that more extensive options than (strict) avoidance of the culprit food are available. In ***Chapter 9***, we systematically reviewed the literature on certain dietary interventions which may affect reaction severity and eliciting dose, allowing subjects with PRFA to consume the offending food. Most research has been performed for hazelnut and apple. Heating of foods cross-reacting with birch or mugwort pollen appears to reduce allergenicity of foods implicated in PRFA, leading to complete prevention of allergic symptoms in 15 to 71% of hazelnut allergic and around 46% of celery allergic subjects. Heating also possibly increases the dose threshold for symptom elicitation in pollen-related hazelnut allergic subjects. Consumption of Santana and possibly Elise apples rather than Golden Delicious apples seems to reduce symptom severity in subjects with birch pollen-related apple allergy. Literature further suggests that oral immunotherapy (OIT) with Golden Delicious apple can reduce the frequency of allergic reactions in birch pollen-related apple allergy, inducing tolerance in 63 to 81% of subjects. None of the studies assessed the degree of sustained unresponsiveness in long-term follow-up, but one study suggested that tolerance may be transient because no significant immunologic changes were observed and one subject relapsed after discontinuing apple consumption during a holiday.[111] Overall, it appears that heat processing, consumption of hypoallergenic cultivars and OIT may diminish or completely prevent allergic reactions in some but not all subjects with PRFA. Other dietary factors which may reduce reaction severity in subjects with PRFA to apple, are consumption outside birch-pollen season,[30] consumption without peel,[225] or consumption of apples stored under certain conditions,[226] though results on the effect of storage duration are contradictory.[227, 228]

Our goal in ***Chapter 9*** was to create an overview of the effect of dietary interventions to increase uniformity in dietary advice given to patients with PRFA. Although a 'one

size fits all' approach does not seem possible in PRFA, treating physicians should be aware of the possible effects of the dietary interventions discussed in the previous paragraph. In order to avoid overly restrictive diets, which can seriously impact quality of life in food allergic patients,[229] those with mild reactions should be encouraged to explore what is and isn't possible.

It would be ideal if we could prevent the development of PRFA in the future. Although the benefits of pollen immunotherapy (subcutaneous or sublingual) for allergic rhinoconjunctivitis are well established,[230] the effects on PRFA are unclear.[112, 184] However, perhaps early pollen immunotherapy in childhood could prevent development of PRFA, and have the potential to seriously reduce the FA burden in birch-endemic regions.

Clinical implications

- As one of the most common types of FA in the European population, the impact of PRFA should be acknowledged, and the condition should be actively managed.
- Heat processing, consumption of hypoallergenic cultivars and OIT may diminish or completely prevent allergic reactions in some but not all subjects with PRFA.
- Patients with PRFA with mild reactions should be encouraged to explore dietary options, to avoid overly restrictive diets.

CONCLUSIONS & FUTURE CONSIDERATIONS

One of the main goals in FA research is to find a way to treat and ultimately prevent FA. In order to sufficiently prioritise and tackle this goal, we need to understand the scale of the problem. Analyses of the EuroPrevall data for this thesis have provided insight into the true scope of European FA, delivering the most comprehensive overview of prevalence of FA across Europe to date, and reliably confirming geographical inhomogeneity (***Chapter 2 and 3***). Both animal and plant source FAs occur frequently in school-age children, whereas plant source FAs dominate in adults. PRFA plays an important role in both generations. Prevalence of FA clearly depends on how FA is defined, the generation under study (children *vs* adults), and geographical location, and reflects public awareness and environmental exposures. This thesis has yielded findings in keeping with both the dual-allergen exposure hypothesis and the microbial exposure hypothesis. Based on our findings in ***Chapter 4*** alongside findings from previous studies, early oral introduction to allergenic foods or purchase of a family dog may be warranted in an attempt to prevent FA development in infancy, provided there are no other contraindications. That said, the majority of postulated (early-life) environmental risk factors were found not to be significantly associated with childhood or adulthood FS (***Chapter 4***). Studies investigating the cause of FA remain a top priority in FA research.[5] Besides mechanistic studies into the underlying immunological mechanisms of FA, prospective longitudinal epidemiological projects have the potential to provide insights into causality that the cross-sectional EuroPrevall project in school-age children and adults could not. The EuroPrevall and Australian HealthNuts birth cohorts could have yielded invaluable information on risk factors if study subjects were prospectively followed until adulthood, but lifelong retention of study subjects is hardly feasible.[231-233] Perhaps a re-evaluation of a sample of the birth and school-age populations during adulthood is still an option, which could also expand knowledge on resolution and onset of FS and FA at a later stage in life.

Another focus area in FA research is diagnostics. FA diagnostic research aims to accurately establish FA (phenotype) without the need for the current gold standard test, (double-blind placebo controlled) oral food challenge. This test not only carries the risk of inducing an anaphylactic reaction, but is time-consuming and costly. Paradoxically, FA diagnostic research is compromised by the limitations of outcome based on DBPCFC, which may underestimate FA due to exclusion or stopping criteria, or overestimate FA due to reporting of subjective symptoms. Although there is currently no way to solve these shortcomings, it is important to be aware of the limitations, and actively consider outcomes based on patient history of truly experienced adverse reactions in combination with IgE sensitisation. In diagnosis of FA, patient history is a key tool available to all physicians.[19] This thesis has scientifically reinforced the value of patient history in FA diagnosis (***Chapter 5***). If

physicians (including GPs and non-allergy specialists) were to address reaction time, reaction reproducibility, presence of OAS and GI-symptoms, and co-existing allergic rhinitis and allergic asthma, in adults presenting with plant source FAs in birch-endemic regions, unnecessary IgE testing and allergist referrals may be reduced by almost 50%. Information from clinical background can also contribute to estimation of reaction severity in peanut and tree nut allergy (***Chapter 7 and 8***). CRD can provide additional information on the risk of mild-to-moderate *versus* severe tree nut allergy (***Chapter 7*** *and Datema et al.*[189]), but cannot replace DBPCFC for diagnosis of hazelnut allergy in adult populations from birch-endemic regions (***Chapter 6***). For peanut allergy, on the other hand, studies have found decision points for IgE to Ara h 2 with 100% positive and/or negative predictive values.[208-210, 214] Measures of diagnostic accuracy in FA are highly dependent on the food concerned and the population involved (e.g. age group, country, setting). The findings in this thesis suggest that FA diagnostics could benefit from further exploration (development and validation) of multivariable models combining *in vivo* and *in vitro* diagnostic measures for FAs in which single diagnostic tests perform insufficiently. In order to achieve the most useful models, it is advisable that future research projects strictly define their study population and domain according to culprit food (e.g. tree nuts *vs* peanut), age group (e.g. infants *vs* school-age children *vs* adults), geographical location (e.g. birch territory *vs* non-birch territory) and setting (e.g. general population *vs* outpatient population). Ideally, reactions during DBPCFC and accidental reactions in real life should both be considered as an outcome measure, either of which could be used for development or validation. Furthermore, ratios of food-specific IgE/total IgE or food-specific IgE/IgG4 may want to be taken into consideration, as well as newer emerging diagnostic test modalities like the Basophil Activation Test (BAT), Mast Cell Activation Test (MAT) and IgE to allergen peptide epitopes.[234-238] For example, the BAT is reported to be potentially useful for assessing clinical relevance of sensitisation to PR-10 proteins, which could help identify the culprit allergen in cases of PRFA.[234, 239-241]

The likelihood of PRFA considerably impacts risk assessment (***Chapter 5 to 8***), and its relevance deserves broader acknowledgement and professional management. Affected patients should explore how restrictive their diet need be, as heat processing or choosing hypoallergenic cultivars may enable consumption of the culprit food (***Chapter 9***). Future research should continue exploring the usefulness of immunotherapy with food and pollen, which respectively have the potential to treat or prevent FA, both key objectives in FA research.

References

1. Burney PG, Potts J, Kummeling I, Mills EN, Clausen M, Dubakiene R, et al. The prevalence and distribution of food sensitization in European adults. Allergy. 2014;69(3):365-71.
2. Yunginger JW, Sweeney KG, Sturner WQ, Giannandrea LA, Teigland JD, Bray M, et al. Fatal food-induced anaphylaxis. JAMA. 1988;260(10):1450-2.
3. Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. N Engl J Med. 1992;327(6):380-4.
4. Wuthrich B. History of food allergy. Chem Immunol Allergy. 2014;100:109-19.
5. Sampson HA. Food allergy: Past, present and future. Allergol Int. 2016;65(4):363-9.
6. Haeusermann T. I can't eat that: The sociology behind the rise in food allergies and intolerances. Current Sociology. 2015;63(3):369-86.
7. Taylor SL, Hefle SL, Bindslev-Jensen C, Atkins FM, Andre C, Bruijnzeel-Koomen C, et al. A consensus protocol for the determination of the threshold doses for allergenic foods: how much is too much? Clin Exp Allergy. 2004;34(5):689-95.
8. Taylor SL, Hefle SL. Food allergen labeling in the USA and Europe. Curr Opin Allergy Clin Immunol. 2006;6(3):186-90.
9. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med. 2015;372(9):803-13.
10. Perkin MR, Logan K, Tseng A, Raji B, Ayis S, Peacock J, et al. Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. N Engl J Med. 2016;374(18):1733-43.
11. Burgess JA, Dharmage SC, Allen K, Koplin J, Garcia-Larsen V, Boyle R, et al. Age at introduction to complementary solid food and food allergy and sensitization: A systematic review and meta-analysis. Clin Exp Allergy. 2019;49(6):754-69.
12. Nwaru BI, Hickstein L, Panesar SS, Muraro A, Werfel T, Cardona V, et al. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. Allergy. 2014;69(1):62-75.
13. Roehr CC, Edenharter G, Reimann S, Ehlers I, Worm M, Zuberbier T, et al. Food allergy and non-allergic food hypersensitivity in children and adolescents. Clin Exp Allergy. 2004;34(10):1534-41.
14. Zuberbier T, Edenharter G, Worm M, Ehlers I, Reimann S, Hantke T, et al. Prevalence of adverse reactions to food in Germany - a population study. Allergy. 2004;59(3):338-45.
15. Nwaru BI, Hickstein L, Panesar SS, Roberts G, Muraro A, Sheikh A, et al. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. Allergy. 2014;69(8):992-1007.
16. Igea JM. The history of the idea of allergy. Allergy. 2013;68(8):966-73.
17. Sampson HA, O'Mahony L, Burks AW, Plaut M, Lack G, Akdis CA. Mechanisms of food allergy. J Allergy Clin Immunol. 2018;141(1):11-9.
18. Nowak-Wegrzyn A, Chatchatee P. Mechanisms of Tolerance Induction. Ann Nutr Metab. 2017;70 Suppl 2:7-24.
19. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol. 2018;141(1):41-58.
20. van Ree R, Hummelshoj L, Plantinga M, Poulsen LK, Swindle E. Allergic sensitization: host-immune factors. Clin Transl Allergy. 2014;4(1):12.
21. van Bilsen JHM, Sienkiewicz-Szlapka E, Lozano-Ojalvo D, Willemsen LEM, Antunes CM, Molina E, et al. Application of the adverse outcome pathway (AOP) concept to structure the available in vivo and in vitro mechanistic data for allergic sensitization to food proteins. Clin Transl Allergy. 2017;7:13.
22. Wambre E, Jeong D. Oral Tolerance Development and Maintenance. Immunol Allergy Clin North Am. 2018;38(1):27-37.
23. Lack G. Epidemiologic risks for food allergy. J Allergy Clin Immunol. 2008;121(6):1331-6.
24. Lack G. Update on risk factors for food allergy. J Allergy Clin Immunol. 2012;129(5):1187-97.
25. Knol EF. Requirements for effective IgE cross-linking on mast cells and basophils. Mol Nutr Food Res. 2006;50(7):620-4.

26. Christensen LH, Holm J, Lund G, Riise E, Lund K. Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. J Allergy Clin Immunol. 2008;122(2):298-304.
27. Gieras A, Focke-Teijkl M, Ball T, Verdino P, Hartl A, Thalhamer J, et al. Molecular determinants of allergen-induced effector cell degranulation. J Allergy Clin Immunol. 2007;119(2):384-90.
28. Celik-Bilgili S, Mehl A, Verstege A, Staden U, Nocon M, Beyer K, et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges. Clin Exp Allergy. 2005;35(3):268-73.
29. Santos AF, James LK, Bahnson HT, Shamji MH, Couto-Francisco NC, Islam S, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. J Allergy Clin Immunol. 2015;135(5):1249-56.
30. Geroldinger-Simic M, Zelniker T, Aberer W, Ebner C, Egger C, Greiderer A, et al. Birch pollen-related food allergy: clinical aspects and the role of allergen-specific IgE and IgG4 antibodies. J Allergy Clin Immunol. 2011;127(3):616-22 e1.
31. James LK, Till SJ. Potential Mechanisms for IgG4 Inhibition of Immediate Hypersensitivity Reactions. Curr Allergy Asthma Rep. 2016;16(3):23.
32. Datema MR, Eller E, Zwinderman AH, Poulsen LK, Versteeg SA, van Ree R, et al. Ratios of specific IgG4 over IgE antibodies do not improve prediction of peanut allergy nor of its severity compared to specific IgE alone. Clin Exp Allergy. 2019;49(2):216-26.
33. Mari A. IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. Int Arch Allergy Immunol. 2002;129(4):286-95.
34. Altmann F. Coping with cross-reactive carbohydrate determinants in allergy diagnosis. Allergo J Int. 2016;25(4):98-105.
35. Hauser M, Roulias A, Ferreira F, Egger M. Panallergens and their impact on the allergic patient. Allergy Asthma Clin Immunol. 2010;6(1):1.
36. McKenna OE, Asam C, Araujo GR, Roulias A, Goulart LR, Ferreira F. How relevant is panallergen sensitization in the development of allergies? Pediatr Allergy Immunol. 2016;27(6):560-8.
37. Jin C, Hantusch B, Hemmer W, Stadlmann J, Altmann F. Affinity of IgE and IgG against cross-reactive carbohydrate determinants on plant and insect glycoproteins. J Allergy Clin Immunol. 2008;121(1):185-90 e2.
38. Pekar J, Ret D, Untersmayr E. Stability of allergens. Mol Immunol. 2018;100:14-20.
39. Verhoeckx KCM, Vissers YM, Baumert JL, Faludi R, Feys M, Flanagan S, et al. Food processing and allergenicity. Food Chem Toxicol. 2015;80:223-40.
40. Mills EN, Sancho AI, Rigby NM, Jenkins JA, Mackie AR. Impact of food processing on the structural and allergenic properties of food allergens. Mol Nutr Food Res. 2009;53(8):963-9.
41. Metcalfe DD. Mast cells and mastocytosis. Blood. 2008;112(4):946-56.
42. da Silva EZ, Jamur MC, Oliver C. Mast cell function: a new vision of an old cell. J Histochem Cytochem. 2014;62(10):698-738.
43. Schwartz SL, Cleyrat C, Olah MJ, Relich PK, Phillips GK, Hlavacek WS, et al. Differential mast cell outcomes are sensitive to FcepsilonRI-Syk binding kinetics. Mol Biol Cell. 2017;28(23):3397-414.
44. Sanderson MP, Gelling SJ, Rippmann JF, Schnapp A. Comparison of the anti-allergic activity of Syk inhibitors with optimized Syk siRNAs in FcepsilonRI-activated RBL-2H3 basophilic cells. Cell Immunol. 2010;262(1):28-34.
45. Nam ST, Park YH, Kim HW, Kim HS, Lee D, Lee MB, et al. Suppression of IgE-mediated mast cell activation and mouse anaphylaxis via inhibition of Syk activation by 8-formyl-7-hydroxy-4-methylcoumarin, 4mu8C. Toxicol Appl Pharmacol. 2017;332:25-31.
46. Perelman B, Adil A, Vadas P. Relationship between platelet activating factor acetylhydrolase activity and apolipoprotein B levels in patients with peanut allergy. Allergy Asthma Clin Immunol. 2014;10(1):20.
47. Gill P, Jindal N, Jagdis A, Vadas P. Platelets in the immune response: Revisiting platelet-activating factor in anaphylaxis. J Allergy Clin Immunol. 2015;135(6):1424-32.
48. Turner PJ, Baumert JL, Beyer K, Boyle RJ, Chan CH, Clark AT, et al. Can we identify patients at risk of life-threatening allergic reactions to food? Allergy. 2016;71(9):1241-55.

49. Vadas P, Gold M, Perelman B, Liss GM, Lack G, Blyth T, et al. Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. N Engl J Med. 2008;358(1):28-35.
50. Summers CW, Pumphrey RS, Woods CN, McDowell G, Pemberton PW, Arkwright PD. Factors predicting anaphylaxis to peanuts and tree nuts in patients referred to a specialist center. J Allergy Clin Immunol. 2008;121(3):632-8 e2.
51. Brown SG, Stone SF, Fatovich DM, Burrows SA, Holdgate A, Celenza A, et al. Anaphylaxis: clinical patterns, mediator release, and severity. J Allergy Clin Immunol. 2013;132(5):1141-9 e5.
52. Kruse S, Mao XQ, Heinzmann A, Blattmann S, Roberts MH, Braun S, et al. The Ile198Thr and Ala379Val variants of plasmatic PAF-acetylhydrolase impair catalytical activities and are associated with atopy and asthma. Am J Hum Genet. 2000;66(5):1522-30.
53. Stafforini DM. PAF acetylhydrolase gene polymorphisms and asthma severity. Pharmacogenomics. 2001;2(3):163-75.
54. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet. 2003;33:245-54.
55. Mitchison N. Polymorphism in regulatory gene sequences. Genome Biol. 2001;2(1):comment2001.1–comment.6.
56. Liu AH, Jaramillo R, Sicherer SH, Wood RA, Bock SA, Burks AW, et al. National prevalence and risk factors for food allergy and relationship to asthma: results from the National Health and Nutrition Examination Survey 2005-2006. J Allergy Clin Immunol. 2010;126(4):798-806 e13.
57. Soost S, Leynaert B, Almqvist C, Edenharter G, Zuberbier T, Worm M. Risk factors of adverse reactions to food in German adults. Clin Exp Allergy. 2009;39(7):1036-44.
58. Melioli G, Marcomini L, Agazzi A, Bazurro G, Tosca M, Rossi GA, et al. The IgE repertoire in children and adolescents resolved at component level: a cross-sectional study. Pediatr Allergy Immunol. 2012;23(5):433-40.
59. Tang ML, Mullins RJ. Food allergy: is prevalence increasing? Intern Med J. 2017;47(3):256-61.
60. Ronmark E, Bjerg A, Perzanowski M, Platts-Mills T, Lundback B. Major increase in allergic sensitization in schoolchildren from 1996 to 2006 in northern Sweden. J Allergy Clin Immunol. 2009;124(2):357-63, 63 e1-15.
61. Ezendam J, Klis vdFR, Loveren vH. Time trends in prevalence of sensitization to milk, egg and peanut in the Netherlands. National Institute for Public Health and the Environment of the Netherlands; 2009. Contract No.: 340350003.
62. Koet LBM, Brand PLP. Increase in atopic sensitization rate among Dutch children with symptoms of allergic disease between 1994 and 2014. Pediatr Allergy Immunol. 2018;29(1):78-83.
63. Venkataraman D, Erlewyn-Lajeunesse M, Kurukulaaratchy RJ, Potter S, Roberts G, Matthews S, et al. Prevalence and longitudinal trends of food allergy during childhood and adolescence: Results of the Isle of Wight Birth Cohort study. Clin Exp Allergy. 2018;48(4):394-402.
64. Alduraywish SA, Lodge CJ, Vicendese D, Lowe AJ, Erbas B, Matheson MC, et al. Sensitization to milk, egg and peanut from birth to 18 years: A longitudinal study of a cohort at risk of allergic disease. Pediatr Allergy Immunol. 2016;27(1):83-91.
65. Savage J, Sicherer S, Wood R. The Natural History of Food Allergy. J Allergy Clin Immunol Pract. 2016;4(2):196-203; quiz 4.
66. Patelis A, Gunnbjornsdottir M, Borres MP, Burney P, Gislason T, Toren K, et al. Natural history of perceived food hypersensitivity and IgE sensitisation to food allergens in a cohort of adults. PLoS One. 2014;9(1):e85333.
67. Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E. Birch pollen allergy in Europe. Allergy. 2019;74(7):1237-48.
68. Vieths S, Scheurer S, Ballmer-Weber B. Current understanding of cross-reactivity of food allergens and pollen. Ann N Y Acad Sci. 2002;964:47-68.
69. Loh W, Tang MLK. The Epidemiology of Food Allergy in the Global Context. Int J Environ Res Public Health. 2018;15(9).
70. Prescott SL, Pawankar R, Allen KJ, Campbell DE, Sinn J, Fiocchi A, et al. A global survey of changing patterns of food allergy burden in children. World Allergy Organ J. 2013;6(1):21.

71. Boye JI. Food allergies in developing and emerging economies: need for comprehensive data on prevalence rates. Clin Transl Allergy. 2012;2(1):25.
72. Sanchez J, Sanchez A. Epidemiologic studies about food allergy and food sensitization in tropical countries. Results and limitations. Allergol Immunopathol (Madr). 2019;47(4):401-8.
73. Sanchez J, Sanchez A. Epidemiology of food allergy in Latin America. Allergol Immunopathol (Madr). 2015;43(2):185-95.
74. Kung SJ, Steenhoff AP, Gray C. Food allergy in Africa: myth or reality? Clin Rev Allergy Immunol. 2014;46(3):241-9.
75. Arakali SR, Green TD, Dinakar C. Prevalence of food allergies in South Asia. Ann Allergy Asthma Immunol. 2017;118(1):16-20.
76. Soller L, Ben-Shoshan M, Harrington DW, Fragapane J, Joseph L, St Pierre Y, et al. Overall prevalence of self-reported food allergy in Canada. J Allergy Clin Immunol. 2012;130(4):986-8.
77. Gupta RS, Springston EE, Warrier MR, Smith B, Kumar R, Pongracic J, et al. The prevalence, severity, and distribution of childhood food allergy in the United States. Pediatrics. 2011;128(1):e9-17.
78. Gupta RS, Springston EE, Smith B, Warrier MR, Pongracic J, Holl JL. Geographic variability of childhood food allergy in the United States. Clin Pediatr (Phila). 2012;51(9):856-61.
79. Taylor-Black S, Wang J. The prevalence and characteristics of food allergy in urban minority children. Ann Allergy Asthma Immunol. 2012;109(6):431-7.
80. McGowan EC, Keet CA. Prevalence of self-reported food allergy in the National Health and Nutrition Examination Survey (NHANES) 2007-2010. J Allergy Clin Immunol. 2013;132(5):1216-9 e5.
81. Vierk KA, Koehler KM, Fein SB, Street DA. Prevalence of self-reported food allergy in American adults and use of food labels. J Allergy Clin Immunol. 2007;119(6):1504-10.
82. Woods RK, Abramson M, Bailey M, Walters EH. International prevalences of reported food allergies and intolerances. Comparisons arising from the European Community Respiratory Health Survey (ECRHS) 1991-1994. Eur J Clin Nutr. 2001;55(4):298-304.
83. Rodriguez-Ortiz PG, Munoz-Mendoza D, Arias-Cruz A, Gonzalez-Diaz SN, Herrera-Castro D, Vidaurri-Ojeda AC. [Epidemiological characteristics of patients with food allergy assisted at Regional Center of Allergies and Clinical Immunology of Monterrey]. Rev Alerg Mex. 2009;56(6):185-91.
84. Marrugo J, Hernandez L, Villalba V. Prevalence of self-reported food allergy in Cartagena (Colombia) population. Allergol Immunopathol (Madr). 2008;36(6):320-4.
85. Silva LA, Silva AF, Ribeiro AC, Silva AO, Vieira FA, Segundo GR. Adult Food Allergy Prevalence: Reducing Questionnaire Bias. Int Arch Allergy Immunol. 2016;171(3-4):261-4.
86. Obeng BB, Amoah AS, Larbi IA, Yazdanbakhsh M, van Ree R, Boakye DA, et al. Food allergy in Ghanaian schoolchildren: data on sensitization and reported food allergy. Int Arch Allergy Immunol. 2011;155(1):63-73.
87. Lunet N, Falcao H, Sousa M, Bay N, Barros H. Self-reported food and drug allergy in Maputo, Mozambique. Public Health. 2005;119(7):587-9.
88. Justin-Temu M, Risha P, Abla O, Massawe A. Incidence, knowledge and health seeking behaviour for perceived allergies at household level: a case study in Ilala district Dar es Salaam Tanzania. East Afr J Public Health. 2008;5(2):90-3.
89. Li J, Ogorodova LM, Mahesh PA, Wang MH, Fedorova OS, Leung TF, et al. Comparative Study of Food Allergies in Children from China, India, and Russia: The EuroPrevall-INCO Surveys. J Allergy Clin Immunol Pract. 2020;8(4):1349-58 e16.
90. Kim M, Lee JY, Jeon HY, Yang HK, Lee KJ, Han Y, et al. Prevalence of Immediate-Type Food Allergy in Korean Schoolchildren in 2015: A Nationwide, Population-based Study. Allergy Asthma Immunol Res. 2017;9(5):410-6.
91. Wu TC, Tsai TC, Huang CF, Chang FY, Lin CC, Huang IF, et al. Prevalence of food allergy in Taiwan: a questionnaire-based survey. Intern Med J. 2012;42(12):1310-5.
92. Ho MH, Lee SL, Wong WH, Ip P, Lau YL. Prevalence of self-reported food allergy in Hong Kong children and teens--a population survey. Asian Pac J Allergy Immunol. 2012;30(4):275-84.

93. Mahesh PA, Wong GW, Ogorodova L, Potts J, Leung TF, Fedorova O, et al. Prevalence of food sensitization and probable food allergy among adults in India: the EuroPrevall INCO study. Allergy. 2016;71(7):1010-9.
94. Sasaki M, Koplin JJ, Dharmage SC, Field MJ, Sawyer SM, McWilliam V, et al. Prevalence of clinic-defined food allergy in early adolescence: The SchoolNuts study. J Allergy Clin Immunol. 2018;141(1):391-8 e4.
95. Panjari M, Koplin JJ, Dharmage SC, Peters RL, Gurrin LC, Sawyer SM, et al. Nut allergy prevalence and differences between Asian-born children and Australian-born children of Asian descent: a state-wide survey of children at primary school entry in Victoria, Australia. Clin Exp Allergy. 2016;46(4):602-9.
96. Koplin JJ, Peters RL, Ponsonby AL, Gurrin LC, Hill D, Tang ML, et al. Increased risk of peanut allergy in infants of Asian-born parents compared to those of Australian-born parents. Allergy. 2014;69(12):1639-47.
97. Allen KJ, Koplin JJ, Ponsonby AL, Gurrin LC, Wake M, Vuillermin P, et al. Vitamin D insufficiency is associated with challenge-proven food allergy in infants. J Allergy Clin Immunol. 2013;131(4):1109-16, 16 e1-6.
98. Graif Y, German L, Livne I, Shohat T. Association of food allergy with asthma severity and atopic diseases in Jewish and Arab adolescents. Acta Paediatr. 2012;101(10):1083-8.
99. Goh DL, Lau YN, Chew FT, Shek LP, Lee BW. Pattern of food-induced anaphylaxis in children of an Asian community. Allergy. 1999;54(1):84-6.
100. Papadopoulos NG, Agache I, Bavbek S, Bilo BM, Braido F, Cardona V, et al. Research needs in allergy: an EAACI position paper, in collaboration with EFA. Clin Transl Allergy. 2012;2(1):21.
101. Cochrane S, Beyer K, Clausen M, Wjst M, Hiller R, Nicoletti C, et al. Factors influencing the incidence and prevalence of food allergy. Allergy. 2009;64(9):1246-55.
102. Ben-Shoshan M, Turnbull E, Clarke A. Food allergy: temporal trends and determinants. Curr Allergy Asthma Rep. 2012;12(4):346-72.
103. McClain S, Bowman C, Fernandez-Rivas M, Ladics GS, Ree R. Allergic sensitization: food- and protein-related factors. Clin Transl Allergy. 2014;4(1):11.
104. Haahtela T. A biodiversity hypothesis. Allergy. 2019;74(8):1445-56.
105. Haahtela T, Holgate S, Pawankar R, Akdis CA, Benjaponpitak S, Caraballo L, et al. The biodiversity hypothesis and allergic disease: world allergy organization position statement. World Allergy Organ J. 2013;6(1):3.
106. Directorate-General for Maritime Affairs and Fisheries (European Commission), EUMOFA. EU consumer habits regarding fishery and aquaculture products. Available from: https://www.efsa.europa.eu/en/foodconsumption/comprehensive-database.
107. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. J Allergy Clin Immunol. 2008;122(5):984-91.
108. Andre F, Andre C, Colin L, Cacaraci F, Cavagna S. Role of new allergens and of allergens consumption in the increased incidence of food sensitizations in France. Toxicology. 1994;93(1):77-83.
109. Tang ML, Martino DJ. Oral immunotherapy and tolerance induction in childhood. Pediatr Allergy Immunol. 2013;24(6):512-20.
110. Berin MC, Mayer L. Can we produce true tolerance in patients with food allergy? J Allergy Clin Immunol. 2013;131(1):14-22.
111. Kopac P, Rudin M, Gentinetta T, Gerber R, Pichler C, Hausmann O, et al. Continuous apple consumption induces oral tolerance in birch-pollen-associated apple allergy. Allergy. 2012;67(2):280-5.
112. Werfel T, Asero R, Ballmer-Weber BK, Beyer K, Enrique E, Knulst AC, et al. Position paper of the EAACI: food allergy due to immunological cross-reactions with common inhalant allergens. Allergy. 2015;70(9):1079-90.
113. Worm M, Jappe U, Kleine-Tebbe J, Schafer C, Reese I, Saloga J, et al. Food allergies resulting from immunological cross-reactivity with inhalant allergens: Guidelines from the German

Society for Allergology and Clinical Immunology (DGAKI), the German Dermatology Society (DDG), the Association of German Allergologists (AeDA) and the Society for Pediatric Allergology and Environmental Medicine (GPA). Allergo J Int. 2014;23(1):1-16.
114. Smits M, Le TM, Welsing P, Houben G, Knulst A, Verhoeckx K. Legume Protein Consumption and the Prevalence of Legume Sensitization. Nutrients. 2018;10(10).
115. Pajno GB, Fernandez-Rivas M, Arasi S, Roberts G, Akdis CA, Alvaro-Lozano M, et al. EAACI Guidelines on allergen immunotherapy: IgE-mediated food allergy. Allergy. 2018;73(4):799-815.
116. Ierodiakonou D, Garcia-Larsen V, Logan A, Groome A, Cunha S, Chivinge J, et al. Timing of Allergenic Food Introduction to the Infant Diet and Risk of Allergic or Autoimmune Disease: A Systematic Review and Meta-analysis. JAMA. 2016;316(11):1181-92.
117. Obbagy JE, English LK, Wong YP, Butte NF, Dewey KG, Fleischer DM, et al. Complementary feeding and food allergy, atopic dermatitis/eczema, asthma, and allergic rhinitis: a systematic review. Am J Clin Nutr. 2019;109(Suppl_7):890S-934S.
118. Koplin JJ, Osborne NJ, Wake M, Martin PE, Gurrin LC, Robinson MN, et al. Can early introduction of egg prevent egg allergy in infants? A population-based study. J Allergy Clin Immunol. 2010;126(4):807-13.
119. Tham EH, Leung DYM. How Different Parts of the World Provide New Insights Into Food Allergy. Allergy Asthma Immunol Res. 2018;10(4):290-9.
120. Perkin MR, Logan K, Bahnson HT, Marrs T, Radulovic S, Craven J, et al. Efficacy of the Enquiring About Tolerance (EAT) study among infants at high risk of developing food allergy. J Allergy Clin Immunol. 2019;144(6):1606-14 e2.
121. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. Peanut allergy. London: Department of Health; 1998.
122. Fiocchi A, Assa'ad A, Bahna S, Adverse Reactions to Foods C, American College of Allergy A, Immunology. Food allergy and the introduction of solid foods to infants: a consensus document. Adverse Reactions to Foods Committee, American College of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol. 2006;97(1):10-20; quiz 1, 77.
123. Ferraro V, Zanconato S, Carraro S. Timing of Food Introduction and the Risk of Food Allergy. Nutrients. 2019;11(5).
124. Muraro A, Halken S, Arshad SH, Beyer K, Dubois AE, Du Toit G, et al. EAACI food allergy and anaphylaxis guidelines. Primary prevention of food allergy. Allergy. 2014;69(5):590-601.
125. Fewtrell M, Bronsky J, Campoy C, Domellof M, Embleton N, Fidler Mis N, et al. Complementary Feeding: A Position Paper by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2017;64(1):119-32.
126. EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA). Appropriate age range for introduction of complementary feeding into an infant's diet. EFSA J. 2019;17(9):e05780.
127. Greenhawt M, Fleischer DM, Chan ES, Venter C, Stukus D, Gupta R, et al. LEAPing through the looking glass: secondary analysis of the effect of skin test size and age of introduction on peanut tolerance after early peanut introduction. Allergy. 2017;72(8):1254-60.
128. Brough HA, Santos AF, Makinson K, Penagos M, Stephens AC, Douiri A, et al. Peanut protein in household dust is related to household peanut consumption and is biologically active. J Allergy Clin Immunol. 2013;132(3):630-8.
129. Trendelenburg V, Ahrens B, Wehrmann AK, Kalb B, Niggemann B, Beyer K. Peanut allergen in house dust of eating area and bed - a risk factor for peanut sensitization? Allergy. 2013;68(11):1460-2.
130. Brough HA, Liu AH, Sicherer S, Makinson K, Douiri A, Brown SJ, et al. Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy. J Allergy Clin Immunol. 2015;135(1):164-70.
131. Fox AT, Sasieni P, du Toit G, Syed H, Lack G. Household peanut consumption as a risk factor for the development of peanut allergy. J Allergy Clin Immunol. 2009;123(2):417-23.
132. Tsakok T, Marrs T, Mohsin M, Baron S, du Toit G, Till S, et al. Does atopic dermatitis cause food allergy? A systematic review. J Allergy Clin Immunol. 2016;137(4):1071-8.

133. Venkataraman D, Soto-Ramirez N, Kurukulaaratchy RJ, Holloway JW, Karmaus W, Ewart SL, et al. Filaggrin loss-of-function mutations are associated with food allergy in childhood and adolescence. J Allergy Clin Immunol. 2014;134(4):876-82 e4.
134. Hill DJ, Sporik R, Thorburn J, Hosking CS. The association of atopic dermatitis in infancy with immunoglobulin E food sensitization. J Pediatr. 2000;137(4):475-9.
135. Sampson HA, Broadbent KR, Bernhisel-Broadbent J. Spontaneous release of histamine from basophils and histamine-releasing factor in patients with atopic dermatitis and food hypersensitivity. N Engl J Med. 1989;321(4):228-32.
136. Brown SJ, Asai Y, Cordell HJ, Campbell LE, Zhao Y, Liao H, et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. J Allergy Clin Immunol. 2011;127(3):661-7.
137. Chan SM, Turcanu V, Stephens AC, Fox AT, Grieve AP, Lack G. Cutaneous lymphocyte antigen and alpha4beta7 T-lymphocyte responses are associated with peanut allergy and tolerance in children. Allergy. 2012;67(3):336-42.
138. Weissler KA, Rasooly M, DiMaggio T, Bolan H, Cantave D, Martino D, et al. Identification and analysis of peanut-specific effector T and regulatory T cells in children allergic and tolerant to peanut. J Allergy Clin Immunol. 2018;141(5):1699-710 e7.
139. Turcanu V, Maleki SJ, Lack G. Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children, and allergic children who acquired tolerance to peanuts. J Clin Invest. 2003;111(7):1065-72.
140. Blom LH, Juel-Berg N, Larsen LF, Hansen KS, Poulsen LK. Circulating allergen-specific TH2 lymphocytes: CCR4(+) rather than CLA(+) is the predominant phenotype in peanut-allergic subjects. J Allergy Clin Immunol. 2018;141(4):1498-501 e5.
141. Dunkin D, Berin MC, Mayer L. Allergic sensitization can be induced via multiple physiologic routes in an adjuvant-dependent manner. J Allergy Clin Immunol. 2011;128(6):1251-8 e2.
142. Kelleher MM, Tran L, Boyle RJ. Prevention of food allergy - skin barrier interventions. Allergol Int. 2020;69(1):3-10.
143. Horimukai K, Morita K, Narita M, Kondo M, Kitazawa H, Nozaki M, et al. Application of moisturizer to neonates prevents development of atopic dermatitis. J Allergy Clin Immunol. 2014;134(4):824-30 e6.
144. Lowe AJ, Su JC, Allen KJ, Abramson MJ, Cranswick N, Robertson CF, et al. A randomized trial of a barrier lipid replacement strategy for the prevention of atopic dermatitis and allergic sensitization: the PEBBLES pilot study. Br J Dermatol. 2018;178(1):e19-e21.
145. Dissanayake E, Tani Y, Nagai K, Sahara M, Mitsuishi C, Togawa Y, et al. Skin Care and Synbiotics for Prevention of Atopic Dermatitis or Food Allergy in Newborn Infants: A 2 x 2 Factorial, Randomized, Non-Treatment Controlled Trial. Int Arch Allergy Immunol. 2019;180(3):202-11.
146. Prusak A, Schlegel-Zawadzka M, Boulay A, Rowe G. Characteristics of the peanut chain in Europe - implications for peanut allergy. Acta Sci Pol Technol Aliment. 2014;13(3):321-33.
147. Beyer K, Morrow E, Li XM, Bardina L, Bannon GA, Burks AW, et al. Effects of cooking methods on peanut allergenicity. J Allergy Clin Immunol. 2001;107(6):1077-81.
148. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. Standardization of food challenges in patients with immediate reactions to foods - position paper from the European Academy of Allergology and Clinical Immunology. Allergy. 2004;59(7):690-7.
149. Hofmann A, Burks AW. Pollen food syndrome: update on the allergens. Curr Allergy Asthma Rep. 2008;8(5):413-7.
150. D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, et al. Allergenic pollen and pollen allergy in Europe. Allergy. 2007;62(9):976-90.
151. Zuidmeer L, van Ree R. Lipid transfer protein allergy: primary food allergy or pollen/food syndrome in some cases. Curr Opin Allergy Clin Immunol. 2007;7(3):269-73.
152. Egger M, Hauser M, Mari A, Ferreira F, Gadermaier G. The role of lipid transfer proteins in allergic diseases. Curr Allergy Asthma Rep. 2010;10(5):326-35.

153. Lauer I, Miguel-Moncin MS, Abel T, Foetisch K, Hartz C, Fortunato D, et al. Identification of a plane pollen lipid transfer protein (Pla a 3) and its immunological relation to the peach lipid-transfer protein, Pru p 3. Clin Exp Allergy. 2007;37(2):261-9.
154. Gao ZS, Yang ZW, Wu SD, Wang HY, Liu ML, Mao WL, et al. Peach allergy in China: a dominant role for mugwort pollen lipid transfer protein as a primary sensitizer. J Allergy Clin Immunol. 2013;131(1):224-6 e1-3.
155. Lombardero M, Garcia-Selles FJ, Polo F, Jimeno L, Chamorro MJ, Garcia-Casado G, et al. Prevalence of sensitization to Artemisia allergens Art v 1, Art v 3 and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid-transfer protein allergens. Clin Exp Allergy. 2004;34(9):1415-21.
156. Wangorsch A, Schülke S, Gadermaier G, Albrecht M, Wallner M, Randow S, et al. LTP cross-reactivity - primary sensitization to mugwort pollen LTP Art v 3, facilitates subsequent sensitisation to peach LTP Pru p 3 in mice. Clin Transl Allergy 2014;4.
157. Fernandez-Rivas M, Bolhaar S, Gonzalez-Mancebo E, Asero R, van Leeuwen A, Bohle B, et al. Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. J Allergy Clin Immunol. 2006;118(2):481-8.
158. Santos A, Van Ree R. Profilins: mimickers of allergy or relevant allergens? Int Arch Allergy Immunol. 2011;155(3):191-204.
159. Buyuktiryaki B, Santos AF. Food allergy severity predictions based on cellular in vitro tests. Expert Rev Mol Diagn. 2020; 20 (7):679-692.
160. Asero R, Monsalve R, Barber D. Profilin sensitization detected in the office by skin prick test: a study of prevalence and clinical relevance of profilin as a plant food allergen. Clin Exp Allergy. 2008;38(6):1033-7.
161. Lopez-Torrejon G, Crespo JF, Sanchez-Monge R, Sanchez-Jimenez M, Alvarez J, Rodriguez J, et al. Allergenic reactivity of the melon profilin Cuc m 2 and its identification as major allergen. Clin Exp Allergy. 2005;35(8):1065-72.
162. Bohle B, Zwolfer B, Heratizadeh A, Jahn-Schmid B, Antonia YD, Alter M, et al. Cooking birch pollen-related food: divergent consequences for IgE- and T cell-mediated reactivity in vitro and in vivo. J Allergy Clin Immunol. 2006;118(1):242-9.
163. Koplin JJ, Dharmage SC, Ponsonby AL, Tang ML, Lowe AJ, Gurrin LC, et al. Environmental and demographic risk factors for egg allergy in a population-based study of infants. Allergy. 2012;67(11):1415-22.
164. Lodge CJ, Allen KJ, Lowe AJ, Hill DJ, Hosking CS, Abramson MJ, et al. Perinatal cat and dog exposure and the risk of asthma and allergy in the urban environment: a systematic review of longitudinal studies. Clin Dev Immunol. 2012;2012:176484.
165. Marrs T, Logan K, Craven J, Radulovic S, Irwin McLean WHA, Lack G, et al. Dog ownership at three months of age is associated with protection against food allergy. Allergy. 2019; 74 (11):2212-2219.
166. Hua X, Goedert JJ, Pu A, Yu G, Shi J. Allergy associations with the adult fecal microbiota: Analysis of the American Gut Project. EBioMedicine. 2016;3:172-9.
167. Noval Rivas M, Burton OT, Wise P, Zhang YQ, Hobson SA, Garcia Lloret M, et al. A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. J Allergy Clin Immunol. 2013;131(1):201-12.
168. Lyons A, O'Mahony D, O'Brien F, MacSharry J, Sheil B, Ceddia M, et al. Bacterial strain-specific induction of Foxp3+ T regulatory cells is protective in murine allergy models. Clin Exp Allergy. 2010;40(5):811-9.
169. Kettleson EM, Adhikari A, Vesper S, Coombs K, Indugula R, Reponen T. Key determinants of the fungal and bacterial microbiomes in homes. Environ Res. 2015;138:130-5.
170. Marrs T, Bruce KD, Logan K, Rivett DW, Perkin MR, Lack G, et al. Is there an association between microbial exposure and food allergy? A systematic review. Pediatr Allergy Immunol. 2013;24(4):311-20 e8.

171. Roduit C, Frei R, Depner M, Schaub B, Loss G, Genuneit J, et al. Increased food diversity in the first year of life is inversely associated with allergic diseases. J Allergy Clin Immunol. 2014;133(4):1056-64.
172. Botha M, Basera W, Facey-Thomas HE, Gaunt B, Gray CL, Ramjith J, et al. Rural and urban food allergy prevalence from the South African Food Allergy (SAFFA) study. J Allergy Clin Immunol. 2019;143(2):662-8 e2.
173. von Hertzen L, Makela MJ, Petays T, Jousilahti P, Kosunen TU, Laatikainen T, et al. Growing disparities in atopy between the Finns and the Russians: a comparison of 2 generations. J Allergy Clin Immunol. 2006;117(1):151-7.
174. Ege MJ, Herzum I, Buchele G, Krauss-Etschmann S, Lauener RP, Roponen M, et al. Prenatal exposure to a farm environment modifies atopic sensitization at birth. J Allergy Clin Immunol. 2008;122(2):407-12, 12 e1-4.
175. Amoah AS, Boakye DA, Yazdanbakhsh M, van Ree R. Influence of Parasitic Worm Infections on Allergy Diagnosis in Sub-Saharan Africa. Curr Allergy Asthma Rep. 2017;17(10):65.
176. Krishna MT, Mahesh PA, Vedanthan P, Moitra S, Mehta V, Christopher DJ. An appraisal of allergic disorders in India and an urgent call for action. World Allergy Organ J. 2020;13(7):100446.
177. Wu Z, Wang L, Tang Y, Sun X. Parasite-Derived Proteins for the Treatment of Allergies and Autoimmune Diseases. Front Microbiol. 2017;8:2164.
178. Fitzsimmons CM, Falcone FH, Dunne DW. Helminth Allergens, Parasite-Specific IgE, and Its Protective Role in Human Immunity. Front Immunol. 2014;5:61.
179. Afifi MA, Jiman-Fatani AA, El Saadany S, Fouad MA. Parasites-allergy paradox: Disease mediators or therapeutic modulators. J Microsc Ultrastruct. 2015;3(2):53-61.
180. Caraballo L, Zakzuk J, Lee BW, Acevedo N, Soh JY, Sanchez-Borges M, et al. Particularities of allergy in the Tropics. World Allergy Organ J. 2016;9:20.
181. Feehley T, Stefka AT, Cao S, Nagler CR. Microbial regulation of allergic responses to food. Semin Immunopathol. 2012;34(5):671-88.
182. Loke P, Lim YA. Helminths and the microbiota: parts of the hygiene hypothesis. Parasite Immunol. 2015;37(6):314-23.
183. Amoah AS, Obeng BB, Larbi IA, Versteeg SA, Aryeetey Y, Akkerdaas JH, et al. Peanut-specific IgE antibodies in asymptomatic Ghanaian children possibly caused by carbohydrate determinant cross-reactivity. J Allergy Clin Immunol. 2013;132(3):639-47.
184. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy. 2014;69(8):1008-25.
185. Sampson HA. Food allergy - accurately identifying clinical reactivity. Allergy. 2005;60 Suppl 79:19-24.
186. Skypala IJ, Venter C, Meyer R, deJong NW, Fox AT, Groetch M, et al. The development of a standardised diet history tool to support the diagnosis of food allergy. Clin Transl Allergy. 2015;5:7.
187. Amlot PL, Kemeny DM, Zachary C, Parkes P, Lessof MH. Oral allergy syndrome (OAS): symptoms of IgE-mediated hypersensitivity to foods. Clin Allergy. 1987;17(1):33-42.
188. Rudders SA, Banerji A, Clark S, Camargo CA, Jr. Age-related differences in the clinical presentation of food-induced anaphylaxis. J Pediatr. 2011;158(2):326-8.
189. Datema MR, van Ree R, Asero R, Barreales L, Belohlavkova S, de Blay F, et al. Component-resolved diagnosis and beyond: Multivariable regression models to predict severity of hazelnut allergy. Allergy. 2018;73(3):549-59.
190. Chong KW, Ruiz-Garcia M, Patel N, Boyle RJ, Turner PJ. Reaction phenotypes in IgE-mediated food allergy and anaphylaxis. Ann Allergy Asthma Immunol. 2020;124(5):473-8.
191. Katelaris CH. Should patients with pollen fruit syndrome be prescribed an automatic epinephrine injector? Curr Opin Allergy Clin Immunol. 2016;16(4):370-4.
192. Smith PK, Hourihane JO, Lieberman P. Risk multipliers for severe food anaphylaxis. World Allergy Organ J. 2015;8(1):30.

193. Pettersson ME, Koppelman GH, Flokstra-de Blok BMJ, Kollen BJ, Dubois AEJ. Prediction of the severity of allergic reactions to foods. Allergy. 2018;73(7):1532-40.
194. National Academies of Sciences E, and Medicine; Health and Medicine Division; Food and Nutrition Board; Committee on Food Allergies. Potential Genetic and Enviromental Determinants of Food Allergy Risk and Possible Prevention Strategies. In: Oria MP, Stallings VA, editors. Finding a Path to Safety in Food Allergy: Assessment of the Global Burden, Causes, Prevention, Management, and Public Policy Washington (DC): National Academies Press; 2016.
195. Rolinck-Werninghaus C, Niggemann B, Grabenhenrich L, Wahn U, Beyer K. Outcome of oral food challenges in children in relation to symptom-eliciting allergen dose and allergen-specific IgE. Allergy. 2012;67(7):951-7.
196. Perry TT, Matsui EC, Conover-Walker MK, Wood RA. Risk of oral food challenges. J Allergy Clin Immunol. 2004;114(5):1164-8.
197. Neuman-Sunshine DL, Eckman JA, Keet CA, Matsui EC, Peng RD, Lenehan PJ, et al. The natural history of persistent peanut allergy. Ann Allergy Asthma Immunol. 2012;108(5):326-31 e3.
198. Benhamou AH, Zamora SA, Eigenmann PA. Correlation between specific immunoglobulin E levels and the severity of reactions in egg allergic patients. Pediatr Allergy Immunol. 2008;19(2):173-9.
199. Calvani M, Cardinale F, Martelli A, Muraro A, Pucci N, Savino F, et al. Risk factors for severe pediatric food anaphylaxis in Italy. Pediatr Allergy Immunol. 2011;22(8):813-9.
200. Flinterman AE, Pasmans SG, Hoekstra MO, Meijer Y, van Hoffen E, Knol EF, et al. Determination of no-observed-adverse-effect levels and eliciting doses in a representative group of peanut-sensitized children. J Allergy Clin Immunol. 2006;117(2):448-54.
201. van Erp FC, Knulst AC, Kentie PA, Pasmans SG, van der Ent CK, Meijer Y. Can we predict severe reactions during peanut challenges in children? Pediatr Allergy Immunol. 2013;24(6):596-602.
202. Zhu J, Pouillot R, Kwegyir-Afful EK, Luccioli S, Gendel SM. A retrospective analysis of allergic reaction severities and minimal eliciting doses for peanut, milk, egg, and soy oral food challenges. Food Chem Toxicol. 2015;80:92-100.
203. Hourihane JO, Grimshaw KE, Lewis SA, Briggs RA, Trewin JB, King RM, et al. Does severity of low-dose, double-blind, placebo-controlled food challenges reflect severity of allergic reactions to peanut in the community? Clin Exp Allergy. 2005;35(9):1227-33.
204. Nowak-Wegrzyn A, Assa'ad AH, Bahna SL, Bock SA, Sicherer SH, Teuber SS, et al. Work Group report: oral food challenge testing. J Allergy Clin Immunol. 2009;123(6 Suppl):S365-83.
205. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, et al. EAACI Molecular Allergology User's Guide. Pediatr Allergy Immunol. 2016;27 Suppl 23:1-250.
206. Soares-Weiser K, Takwoingi Y, Panesar SS, Muraro A, Werfel T, Hoffmann-Sommergruber K, et al. The diagnosis of food allergy: a systematic review and meta-analysis. Allergy. 2014;69(1):76-86.
207. DunnGalvin A, Daly D, Cullinane C, Stenke E, Keeton D, Erlewyn-Lajeunesse M, et al. Highly accurate prediction of food challenge outcome using routinely available clinical data. J Allergy Clin Immunol. 2011;127(3):633-9 e1-3.
208. Klemans RJ, Otte D, Knol M, Knol EF, Meijer Y, Gmelig-Meyling FH, et al. The diagnostic value of specific IgE to Ara h 2 to predict peanut allergy in children is comparable to a validated and updated diagnostic prediction model. J Allergy Clin Immunol. 2013;131(1):157-63.
209. Klemans RJ, Broekman HC, Knol EF, Bruijnzeel-Koomen CA, Otten HG, Pasmans SG, et al. Ara h 2 is the best predictor for peanut allergy in adults. J Allergy Clin Immunol Pract. 2013;1(6):632-8 e1.
210. Kansen HM, Erp v, FC, Knulst AC, Ehlers AM, Lyons SA, Knol EF, et al. Accurate prediction of peanut allergy in on third of adults using a validated Ara h 2 cutoff. 202; S2213-2198(20)31245-9.
211. Luttkopf D, Muller U, Skov PS, Ballmer-Weber BK, Wuthrich B, Skamstrup Hansen K, et al. Comparison of four variants of a major allergen in hazelnut (Corylus avellana) Cor a 1.04 with the major hazel pollen allergen Cor a 1.01. Mol Immunol. 2002;38(7):515-25.

212. Mittag D, Akkerdaas J, Ballmer-Weber BK, Vogel L, Wensing M, Becker WM, et al. Ara h 8, a Bet v 1-homologous allergen from peanut, is a major allergen in patients with combined birch pollen and peanut allergy. J Allergy Clin Immunol. 2004;114(6):1410-7.
213. Aalberse RC. Structural biology of allergens. J Allergy Clin Immunol. 2000;106(2):228-38.
214. Flores Kim J, McCleary N, Nwaru BI, Stoddart A, Sheikh A. Diagnostic accuracy, risk assessment, and cost-effectiveness of component-resolved diagnostics for food allergy: A systematic review. Allergy. 2018;73(8):1609-21.
215. Masthoff LJ, Mattsson L, Zuidmeer-Jongejan L, Lidholm J, Andersson K, Akkerdaas JH, et al. Sensitization to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. J Allergy Clin Immunol. 2013;132(2):393-9.
216. Beyer K, Grabenhenrich L, Hartl M, Beder A, Kalb B, Ziegert M, et al. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. Allergy. 2015;70(1):90-8.
217. Eller E, Mortz CG, Bindslev-Jensen C. Cor a 14 is the superior serological marker for hazelnut allergy in children, independent of concomitant peanut allergy. Allergy. 2016;71(4):556-62.
218. Dang TD, Tang M, Choo S, Licciardi PV, Koplin JJ, Martin PE, et al. Increasing the accuracy of peanut allergy diagnosis by using Ara h 2. J Allergy Clin Immunol. 2012;129(4):1056-63.
219. Ebisawa M, Moverare R, Sato S, Maruyama N, Borres MP, Komata T. Measurement of Ara h 1-, 2-, and 3-specific IgE antibodies is useful in diagnosis of peanut allergy in Japanese children. Pediatr Allergy Immunol. 2012;23(6):573-81.
220. Nicolaou N, Murray C, Belgrave D, Poorafshar M, Simpson A, Custovic A. Quantification of specific IgE to whole peanut extract and peanut components in prediction of peanut allergy. J Allergy Clin Immunol. 2011;127(3):684-5.
221. Nicolaou N, Poorafshar M, Murray C, Simpson A, Winell H, Kerry G, et al. Allergy or tolerance in children sensitized to peanut: prevalence and differentiation using component-resolved diagnostics. J Allergy Clin Immunol. 2010;125(1):191-7 e1-13.
222. Nilsson C, Berthold M, Mascialino B, Orme M, Sjolander S, Hamilton R. Allergen components in diagnosing childhood hazelnut allergy: Systematic literature review and meta-analysis. Pediatr Allergy Immunol. 2020;31(2):186-96.
223. Lyons SA, Dijk AMV, Knulst AC, Alquati E, Le TM, Os-Medendorp HV. Dietary Interventions in Pollen-Related Food Allergy. Nutrients. 2018;10(10).
224. McWilliam VL, Perrett KP, Dang T, Peters RL. Prevalence and natural history of tree nut allergy. Ann Allergy Asthma Immunol. 2020; 124 (5):466-472.
225. Fernandez-Rivas M, Cuevas M. Peels of Rosaceae fruits have a higher allergenicity than pulps. Clin Exp Allergy. 1999;29(9):1239-47.
226. Bolhaar ST, van de Weg WE, van Ree R, Gonzalez-Mancebo E, Zuidmeer L, Bruijnzeel-Koomen CA, et al. In vivo assessment with prick-to-prick testing and double-blind, placebo-controlled food challenge of allergenicity of apple cultivars. J Allergy Clin Immunol. 2005;116(5):1080-6.
227. Vlieg-Boerstra BJ, van de Weg WE, van der Heide S, Kerkhof M, Arens P, Heijerman-Peppelman G, et al. Identification of low allergenic apple cultivars using skin prick tests and oral food challenges. Allergy. 2011;66(4):491-8.
228. Kiewning D, Schmitz-Eiberger M. Effects of long-term storage on Mal d 1 content of four apple cultivars with initial low Mal d 1 content. J Sci Food Agric. 2014;94(4):798-802.
229. Antolin-Amerigo D, Manso L, Caminati M, de la Hoz Caballer B, Cerecedo I, Muriel A, et al. Quality of life in patients with food allergy. Clin Mol Allergy. 2016;14:4.
230. Roberts G, Pfaar O, Akdis CA, Ansotegui IJ, Durham SR, Gerth van Wijk R, et al. EAACI Guidelines on Allergen Immunotherapy: Allergic rhinoconjunctivitis. Allergy. 2018;73(4):765-98.
231. Keil T, McBride D, Grimshaw K, Niggemann B, Xepapadaki P, Zannikos K, et al. The multinational birth cohort of EuroPrevall: background, aims and methods. Allergy. 2010;65(4):482-90.
232. Osborne NJ, Koplin JJ, Martin PE, Gurrin LC, Thiele L, Tang ML, et al. The HealthNuts population-based study of paediatric food allergy: validity, safety and acceptability. Clin Exp Allergy. 2010;40(10):1516-22.

233. Koplin JJ, Wake M, Dharmage SC, Matheson M, Tang ML, Gurrin LC, et al. Cohort Profile: The HealthNuts Study: Population prevalence and environmental/genetic predictors of food allergy. Int J Epidemiol. 2015;44(4):1161-71.
234. Santos AF, Brough HA. Making the Most of In Vitro Tests to Diagnose Food Allergy. J Allergy Clin Immunol Pract. 2017;5(2):237-48.
235. Hemmings O, Kwok M, McKendry R, Santos AF. Basophil Activation Test: Old and New Applications in Allergy. Curr Allergy Asthma Rep. 2018;18(12):77.
236. Bahri R, Custovic A, Korosec P, Tsoumani M, Barron M, Wu J, et al. Mast cell activation test in the diagnosis of allergic disease and anaphylaxis. J Allergy Clin Immunol. 2018;142(2):485-96 e16.
237. Flinterman AE, Knol EF, Lencer DA, Bardina L, den Hartog Jager CF, Lin J, et al. Peanut epitopes for IgE and IgG4 in peanut-sensitized children in relation to severity of peanut allergy. J Allergy Clin Immunol. 2008;121(3):737-43 e10.
238. Ehlers AM, Blankestijn MA, Knulst AC, Klinge M, Otten HG. Can alternative epitope mapping approaches increase the impact of B-cell epitopes in food allergy diagnostics? Clin Exp Allergy. 2019;49(1):17-26.
239. Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ. Flow cytometric analysis of in vitro activated basophils, specific IgE and skin tests in the diagnosis of pollen-associated food allergy. Cytometry B Clin Cytom. 2005;64(1):28-33.
240. Glaumann S, Nilsson C, Johansson SG, Asarnoj A, Wickman M, Borres MP, et al. Evaluation of basophil allergen threshold sensitivity (CD-sens) to peanut and Ara h 8 in children IgE-sensitized to Ara h 8. Clin Mol Allergy. 2015;13(1):5.
241. Wolbing F, Kunz J, Kempf WE, Grimmel C, Fischer J, Biedermann T. The clinical relevance of birch pollen profilin cross-reactivity in sensitized patients. Allergy. 2017;72(4):562-9.

Chapter 11

English summary
Nederlandse samenvatting

English summary

A food allergy is an abnormal immune reaction to a normally harmless food. Typical food allergy symptoms can vary from just a mild oral itch to life-threatening shortness of breath or loss of blood pressure after ingestion of the culprit food. The immune reaction is mediated by IgE type antibodies. The presence of IgE antibodies to a specific food in the blood is called *sensitisation*. Only when sensitisation is accompanied by typical allergy symptoms is it referred to as a food allergy.

The scientific knowledge with regard to food allergy has increased substantially over the course of time, but there are still many unknowns. The studies described in the previous chapters aimed to expand the knowledge on food allergy in Europe on both a population and a patient level. As the title of this thesis indicates, the studies focused mainly on the following subjects: prevalence (how often does food allergy occur?), predictors (which factors predict the development, the presence or the severity of a food allergy?), and patient profiles (what characterises food allergic patients?). The main findings were re-explored in the final chapter, the general discussion, to better understand geographical variation and to evaluate implications for food allergy diagnostics and management in clinical practice.

Food allergy in the general population: prevalence and potential risk factors
From 2005 to 2009, a large-scale research project on food allergy was conducted throughout Europe: the EuroPrevall project. Information regarding food adverse reactions and allergic predisposition was collected according to a standardised approach from children and adults in eight countries. Participants were asked to report whether they experienced symptoms to 24 relevant foods: cow's milk, hen's egg, fish, shrimp, hazelnut, walnut, peach, apple, kiwi, melon, banana, tomato, celery, carrot, peanut, soybean, lentils, wheat, buckwheat, corn, sesame seed, mustard seed, sunflower seed and poppy seed. A blood test was then performed in each participant to check for IgE sensitisation to those same 24 foods. Based on these data, we determined prevalence estimates for 'self-reported food allergy', 'food sensitisation', and clinically manifest 'probable food allergy' (defined as self-reported symptoms with matching IgE sensitisation) in ***Chapter 2 and 3***. This led to the most extensive overviews of the prevalence of food allergy in Europe to date.

The results confirmed that prevalence of food allergy based on self-report substantially overestimates prevalence of probable food allergy. This means that many individuals report symptoms after ingestion of a particular food, without having an actual food allergy. We also observed that prevalence estimates varied considerably from country to country across Europe. In school-age children (7-10 years), prevalence of self-reported food allergy ranged from 7% in Greece to 25% in Poland, the prevalence of food sensitisation from 11% in Iceland to 29% in

Switzerland, and the prevalence of probable food allergy from 2% in Iceland to 6% in Poland (***Chapter 2***). In adults (20-54 years), prevalence estimates ranged from less than 1% in Lithuania to 19% in Spain for self-reported food allergy, from 7% in Iceland to 24% in Switzerland for food sensitisation, and from less than 1% in Greece to 6% in Switzerland for probable food allergy (***Chapter 3***). Both animal source foods (especially milk and egg) and plant source foods (like nuts, fruits and vegetables) were found to be important sources of food allergy in children, whereas plant source food allergies clearly dominated in adults.

The foods that were mostly responsible for food sensitisation and allergy differed per country. Sensitisation and subsequent allergy to a specific food can be directly induced by the culprit food (primary food allergy), or can be the result of cross-reactivity with another food or pollen (secondary food allergy). Geographical prevalence variations were clearly related to local pollen exposure and presumably also to local dietary preferences. In Northern and Central Europe, the prevalence of hazelnut, apple, peach, kiwi, celery and carrot allergy was high. This is due to the abundance of birch trees in these regions. Individuals who are allergic to birch pollen often have a (usually mild) food allergy to nuts, *Rosaceae* fruits, and certain vegetables, because of cross-reactivity between similar proteins (PR-10 proteins) in birch and aforementioned foods. In Mediterranean countries, we also observed many allergies to peach and kiwi, and relatively more allergies to melon, banana, walnut, lentils and sunflower seeds than in more Northerly regions. Birch trees hardly grow in the Mediterranean. Other cross-reactive proteins than PR-10 proteins, such as profilin in grass pollen or lipid transfer proteins in peach, are a more likely cause of secondary food allergies in the Mediterranean parts of Europe. The thought that local dietary habits also affect differences in food allergy prevalence was supported by the finding that fish and shrimp allergies occurred relatively most often in countries where these foods are consumed most - in Spain, Greece and Iceland.

Besides pollen and frequency of consumption, it seems likely that other environmental exposures also influence the development and ultimately the prevalence of food sensitisation and food allergy. In **Chapter 4**, we therefore investigated how various (early-life) exposures are related to food sensitisation at school-age and in adulthood. Having a pet dog at an early age was found to be associated with lower likelihood of food sensitisation at a later age. Other postulated early-life exposures appeared to have a more limited impact on food sensitisation later in life. A tentative protective effect was observed for having multiple (older) siblings, attending day care, sharing a bedroom, growing up on a farm, or early introduction of solid foods. There are several hypotheses that can help understand these observations. Greater and more diverse exposure to micro-organisms may be a reason why having a pet dog or a large family leads to a smaller chance of developing a food allergy. Another well-known hypothesis, for which considerable

evidence has been gathered in recent years, claims that early exposure of the digestive tract to large amounts of a specific food allergen, as is the case with early introduction of solid foods in infants, prevents food allergy. Based on the current knowledge, guidelines with regard to infant feeding already advise not to delay introduction of allergenic foods.

Food allergy in presenting patients: prediction, patient profiles and pollen

Food allergy affects up to 6% of the general population in some parts of Europe. However, an even larger part of the population wrongly labels a food adverse reaction as a food allergy. Accurate diagnosis of food allergy in patients presenting with symptoms is key to drawing correct conclusions and subsequently suggesting suitable dietary restrictions and emergency medication.

The 'gold standard' for affirming or excluding food allergy is the double-blind placebo-controlled food challenge (DBPCFC). During this two-day examination, patients ingest the culprit food in gradually increasing doses one day, and a placebo the other day, both of which are unrecognisably incorporated into porridge or cake. Elicited reactions provide insight into the presence or absence of a food allergy, as well as information on severity and eliciting dose. Even so, there are noteworthy downsides to this test. It is burdensome for the patients, costs a considerable amount of time and money, and can only be conducted in specialised clinics. Accurate alternative diagnostic techniques that can reduce the number of required food challenges are therefore urgently needed.

One tool available to all health care workers is patient history. Current European guidelines state that health professionals should inquire how soon after ingestion the patient's reaction occurs, whether the reaction is recurrently elicited by the same food, which symptoms are experienced, and if the patient has any other allergic conditions. ***Chapter 5*** provided scientific evidence in support of these recommendations for the first time. This research showed that information available from patient history can be used to accurately distinguish presence from absence of food allergy, especially in the case of plant source food allergy. Prediction was found to be even more accurate in adults than in children. Oral allergy symptoms elicited by the food (itch or burning sensation in the mouth and/or throat) and having co-existing allergic rhinitis (e.g. hay fever) were found to be particularly strong predictors of food allergy. Gastrointestinal symptoms (vomiting or diarrhoea) made a food allergy less likely. Our findings reveal that approximately half of the adults presenting with food adverse reactions could be correctly classified as non-allergic based on information available from patient history. It is important to realise that most participants were from birch territory and that results may therefore mainly be useful in birch-endemic regions.

After obtaining patient history, determining food sensitisation is a crucial subsequent step in the food allergy diagnostic work-up. Sensitisation can be determined by skin prick testing or blood testing. The blood tests can quantify the amount of IgE to the whole food (extract) or to specific allergenic proteins in the food (components). The latter tests are referred to as 'component-resolved diagnostics' (CRD) and are increasingly applied in daily practice. For peanut, it has become clear that IgE levels to allergen component Ara h 2 and/or Ara h 6 can accurately predict peanut allergy in both children and adults. As regards hazelnut, several studies have demonstrated that IgE to hazelnut allergen components Cor a 9 and Cor a 14 can accurately predict hazelnut allergy in children. However, in ***Chapter 6*** we found that the level of IgE to hazelnut extract and hazelnut components Cor a 1, 8, 9 and 14 could not discriminate between presence and absence of hazelnut allergy in Dutch adults. The lack of predictive value is probably mainly due to the high prevalence of Cor a 1 sensitisation in Dutch adults. Hazelnut component Cor a 1 is cross-reactive with the major birch pollen allergen Bet v 1. Sensitisation to Cor a 1 can often, but does not necessarily, lead to allergic symptoms to hazelnut. We concluded that adult individuals from areas with considerable exposure to birch currently remain dependent on food challenge for accurate diagnosis of hazelnut allergy.

To date, most studies demonstrate that CRD cannot predict *severity* of a food allergy. There are indications that combining information from patient history with results from skin prick tests and blood tests can lead to better estimation of the risk of a severe reaction. Such combinations provide insight into the characteristics of patients with a mild or a severe food allergy. A recent study evaluated data of paediatric and adult EuroPrevall participants who visited an allergy outpatient clinic with a suspected hazelnut allergy. This study presented a model combining information on atopic dermatitis, hay fever, IgE to walnut extract and IgE to hazelnut component Cor a 14 that could accurately predict the severity of hazelnut allergy.

In ***Chapter 7 and 8*** of this thesis, the same outpatient data were used to investigate whether similar combination models could be used to estimate the severity of respectively walnut and peanut allergy. First, we evaluated the predictive value of the patients' clinical background, which consisted of information available from patient history, such as the age of onset of symptoms or the presence of pollen allergy. These analyses showed that birch pollen allergy was associated with a mild walnut or peanut allergy. Clinical background characteristics that were related to a severe walnut or peanut allergy included a familial disposition to allergy, having atopic dermatitis, and symptoms elicited by skin contact with the culprit food. We also found differences between predictors for severity of walnut and peanut allergy. Allergy to mugwort pollen (a type of weed) was predictive of severe walnut allergy, and allergy to house dust mite was predictive of severe peanut allergy. For hazelnut (in an earlier EuroPrevall study), walnut and peanut, we observed that a combination

of clinical background characteristics led to better discrimination between mild and severe allergies than individual tests for measurement of IgE sensitisation (skin prick tests and blood tests). That said, CRD test results were found to improve prediction of severity of hazelnut and walnut allergy when added to clinical background information. This was not the case for peanut allergy. It was noteworthy that most variables associated with a mild allergy to hazelnut, walnut or peanut, indicated cross-reactive sensitisation through pollen. Because pollen-related food allergy occurs frequently in Europe and is less clinically relevant for peanut than for tree nut allergy, this could explain why CRD contributes more to prediction of severity of tree nut allergy. We concluded from ***Chapter 7 and 8*** that a patient's clinical background contributes more to prediction of severity of tree nut and peanut allergy than results from individual skin prick tests or blood tests, but that models combining clinical background variables with measures of IgE sensitisation do improve prediction of severity of tree nut allergy. Such combination models have the potential to support decision making in future clinical practice, reducing the need for food challenges.

The findings in ***Chapter 2 to 8*** of this thesis corroborate the frequent occurrence and clinical relevance of (mainly birch) pollen-related food allergy in Europe. As mentioned before, pollen-related food allergy generally presents with mild (oral allergy) symptoms. The proteins responsible for this condition are usually broken down by heat processing or digestion. For this reason, strict avoidance of the culprit food is not always necessary in these patients, and other dietary advice or treatments may be more suited to this type of food allergy. The literature review in ***Chapter 9*** was dedicated to this subject. We found that heat processing of the culprit food, consumption of hypoallergenic cultivars, or oral immunotherapy with the culprit food, can contribute to prevention or reduction of pollen-related food allergic reactions in some patients. Patients with mild symptoms should therefore be encouraged to actively explore their personal dietary options.

Notes for future research

Chapter 10 discussed this thesis' most important findings in the context of other currently available literature on food allergy, which led to recommendations for future research.

Projects focusing on the causes of food allergy are and will remain a top priority. Prospective studies in which subjects are followed until they develop a food allergy could provide considerable evidence with regard to causality. Crucial information on risk factors could be obtained if study participants of the European EuroPrevall or Australian Healthnuts birth and paediatric cohorts were to be re-evaluated during adulthood. This could expand knowledge regarding the development or resolution of food sensitisation and food allergy at a later age.

The findings in this thesis also show that food allergy diagnostics could benefit from further exploration of prediction models combining clinical background with measures of IgE sensitisation. To arrive at the most useful models, future research projects should strictly define their study population and domain based on the culprit food (e.g. tree nuts *vs* peanut), age group (e.g. infants *vs* school-age children *vs* adults) and setting (e.g. general population *vs* outpatient population). Ideally, these studies should take both reactions during challenge testing and spontaneously occurring reactions in daily life into account, because symptoms observed during food challenge often differ from those of a spontaneous allergic reaction. Furthermore, analyses could be enriched by adding upcoming diagnostic techniques, such as ratios of specific IgE to IgG_4 (a type of antibody that can block allergic immune responses), tests for detecting presence of IgE to specific epitopes (the places where antibodies can bind to the allergen), or the basophil and mast cell activation tests (tests that measure the activity of cells releasing the mediators responsible for allergic symptoms). As regards pollen-related food allergy, future research should further explore the effectiveness of immunotherapy with food and pollen, as both have the potential to treat or prevent food allergy.

Nederlandse samenvatting

Een voedselallergie betreft een abnormale reactie van het afweersysteem op een 'onschuldig' voedingsmiddel. Klachten die kunnen passen bij een voedselallergie lopen uiteen van slechts milde jeuk in de mond tot levensbedreigende benauwdheid of bloeddrukdaling na inname van het verdachte voedingsmiddel. Een dergelijke reactie wordt teweeggebracht door antistoffen van het type IgE. De aanwezigheid van IgE antistoffen tegen een specifiek voedingsmiddel in het bloed wordt *sensibilisatie* genoemd. Pas als sensibilisatie gepaard gaat met typische klachten, spreken we van een voedselallergie.

In de loop der jaren is de wetenschappelijke kennis over voedselallergie enorm toegenomen, maar er blijft veel onduidelijk. De onderzoeken uit dit proefschrift hadden als doel de kennis over voedselallergie in Europa te vergroten, zowel op het niveau van de algemene bevolking als op het niveau van de individuele patiënt. Zoals de titel van dit proefschrift aangeeft, richtten de onderzoeken zich vooral op de volgende onderwerpen: prevalentie (hoe vaak komt voedselallergie voor?), predictoren (welk factoren voorspellen het ontstaan, de aanwezigheid, of de ernst van een voedselallergie?) en patiëntprofielen (wat karakteriseert patiënten met een voedselallergie?). In het laatste hoofdstuk, de discussie, werden de bevindingen onder de loep genomen om geografische verschillen beter te begrijpen, en om een vertaalslag te maken naar betere diagnostiek en omgangsadviezen in de praktijk.

Voedselallergie in de bevolking: prevalentie en potentiële risicofactoren
Tussen 2005 en 2009 werd onder de naam 'EuroPrevall' een grootschalig onderzoeksproject naar voedselallergie in Europa uitgevoerd. Onderzoekers verzamelden volgens een gestandaardiseerde aanpak informatie over allergische aanleg en reacties op voedingsmiddelen bij kinderen en volwassenen in acht landen. Aan deelnemers werd gevraagd of zij klachten ervaarden na het eten van 24 belangrijke voedingsmiddelen: koemelk, kippenei, vis, garnaal, hazelnoot, walnoot, perzik, appel, kiwi, meloen, banaan, tomaat, selderij, wortel, pinda, soja, linzen, tarwe, boekweit, mais, sesamzaad, mosterdzaad, zonnebloempitten en maanzaad. Voor diezelfde 24 voedingsmiddelen werd door middel van een bloedtest gekeken of deelnemers IgE-antistoffen hadden, ofwel gesensibiliseerd waren. Op basis van deze gegevens bepaalden wij in ***Hoofdstuk 2 en 3*** de prevalentie van 'zelf-gerapporteerde voedselallergie', 'voedselsensibilisatie' en 'klinisch bevestigde voedselallergie' (gedefinieerd als zelf-gerapporteerde allergische symptomen met bijpassende sensibilisatie). Dit resulteerde in de meest omvangrijke overzichten van de prevalentie van voedselallergie in Europa tot nu toe.

De prevalentie van voedselallergie gebaseerd op zelfrapportage bleek een behoorlijke overschatting te geven ten opzichte van de prevalentie van klinisch

bevestigde voedselallergie. Dit betekent dat veel mensen klachten rapporteerden na het eten van voedingsmiddelen, zonder dat er daadwerkelijk sprake was van een voedselallergie. Ook was er grote variatie in prevalentieschattingen tussen verschillende Europese landen. Bij kinderen van schoolleeftijd (7-10 jaar) varieerde de prevalentie van zelf-gerapporteerde voedselallergie van 7% in Griekenland tot 25% in Polen, de prevalentie van voedselsensibilisatie van 11% in IJsland tot 29% in Zwitserland, en de prevalentie van klinisch bevestigde voedselallergie van 2% in IJsland tot 6% in Polen (***Hoofdstuk 2***). Bij volwassenen (20-54 jaar) varieerde de prevalentie van minder dan 1% in Litouwen tot 19% in Spanje voor zelf-gerapporteerde voedselallergie, van 7% in IJsland tot 24% in Zwitserland voor voedselsensibilisatie, en van minder dan 1% in Griekenland tot 6% in Zwitserland voor klinisch bevestigde voedselallergie (***Hoofdstuk 3***). Zowel dierlijke (vooral melk en ei) als plantaardige voedingsmiddelen (zoals noten, fruit en groenten) bleken belangrijke bronnen van voedselallergie bij kinderen, terwijl plantaardige voedingsmiddelen duidelijk de overhand hadden bij volwassenen.

De voedingsmiddelen die het vaakst verantwoordelijk waren voor voedselsensibilisatie en -allergie, verschilden per land. Sensibilisatie en daaropvolgende allergie voor een bepaald voedingsmiddel kunnen direct door het betreffende voedingsmiddel veroorzaakt worden (primaire voedselallergie), of komen door een kruisreactie met andere voedingsmiddelen of pollen (secundaire voedselallergie). Geografische verschillen in prevalentie waren duidelijk gerelateerd aan lokale blootstelling aan pollen en vermoedelijk ook aan lokale dieetvoorkeuren. In Noord- en Centraal-Europa was de prevalentie van hazelnoot-, appel-, perzik-, kiwi-, selderij- en wortelallergie hoog. Dit komt doordat hier veel berken groeien. Mensen die allergisch zijn voor berk hebben geregeld een (meestal milde) voedselallergie voor noten, roosfruit, en sommige groenten door een kruisreactie op basis van een soortgelijk eiwit (het PR-10 eiwit) in berk en de betreffende voedingsmiddelen. In Mediterrane landen zagen we ook veel allergieën voor perzik en kiwi, en relatief vaker allergieën voor onder andere meloen, banaan, walnoot, linzen en zonnebloempitten dan in de Noorderlijkere regio's. In Mediterrane gebieden komt berk nauwelijks voor. Andere kruisreagerende eiwitten dan PR-10 eiwitten, zoals profiline in graspollen of 'lipid transfer proteins' in perzik, zijn eerder verantwoordelijk voor secundaire voedselallergieën in Mediterrane landen. Ook plaatselijke dieetgewoonten lijken te leiden tot verschil in prevalentie; vis- en garnaalallergieën kwamen vaker voor in landen waar deze voedingsmiddelen het meest geconsumeerd worden - in Spanje, Griekenland en IJsland.

Behalve pollenblootstelling en dieetvoorkeuren, is het aannemelijk dat ook andere omgevingsfactoren invloed hebben op de ontwikkeling en prevalentie van voedselsensibilisatie en -allergie. ***Hoofdstuk 4*** onderzocht daarom hoe (vroege) blootstellingen samenhingen met voedselsensibilisatie op schoolleeftijd en

volwassen leeftijd. Zo ging het hebben van een hond op jonge leeftijd samen met een kleinere kans op voedselsensibilisatie op latere leeftijd, wat in overeenstemming was met eerdere studies. Andere geëvalueerde blootstellingen lieten een minder duidelijk verband met latere voedselsensibilisatie zien. Mogelijk beschermt ook het hebben van meerdere (oudere) broers of zussen, het bezoeken van een kinderdagverblijf, het delen van een slaapkamer, het opgroeien op een boerderij of de vroege introductie van vaste voeding tegen voedselallergie. Er bestaan verschillende hypothesen ter verklaring van deze observaties. Het hebben van een hond of een groot gezin zou door een grotere of gevarieerdere blootstelling aan micro-organismen leiden tot een kleinere kans op allergie. Een andere hypothese waar inmiddels veel bewijs voor is gevonden, is dat vroege blootstelling van het maagdarmkanaal aan een allergeen, zoals bij vroege introductie van vaste voeding bij baby's, voedselallergie voorkomt. Op basis van de bestaande kennis adviseren huidige richtlijnen al om introductie van allergene voedingsmiddelen bij zuigelingen niet te lang uit te stellen.

Voedselallergie in de individuele patiënt: predictie, patiëntprofielen en pollen
Voedselallergie treft tot wel 6% van de algemene bevolking in sommige delen van Europa. Echter, een nog veel groter deel meent onterecht een voedselallergie te hebben. Nauwkeurige diagnostiek van voedselallergie bij patiënten die zich presenteren met klachten is van groot belang om de juiste conclusies te trekken en zo gepaste dieetadviezen te kunnen geven en medicatie voor te kunnen schrijven.

De 'gouden standaard' voor het vaststellen dan wel uitsluiten van voedselallergie is de dubbelblinde placebo-gecontroleerde provocatietest. Bij deze tweedaagse test eten patiënten de ene dag in geleidelijk oplopende dosering het verdachte voedingsmiddel en de andere dag een placebo, welke beide onherkenbaar verwerkt zijn in bijvoorbeeld een pap of cake. Eventuele reacties geven inzicht in de aan- of afwezigheid van een voedselallergie, alsook in de ernst en de uitlokkende dosering. Desalniettemin zitten er ook nadelen aan dit onderzoek. De test is belastend voor de patiënt, kost veel tijd en geld, en is alleen uit te voeren in speciaal daarvoor ingerichte klinieken. Nauwkeurige alternatieve diagnostische technieken waarmee het aantal voedselprovocaties verminderd kan worden, zijn daarom hoognodig.

Een instrument dat alle zorgverleners tot hun beschikking hebben, is de anamnese – het gesprek met de patiënt. De huidige Europese richtlijnen stellen dat het van belang is om de patiënt te vragen hoe snel een reactie optreedt, of de reactie herhaaldelijk optreedt op hetzelfde voedingsmiddel, welke symptomen zich voordoen, en of er bijkomende allergische aandoeningen zijn. In ***Hoofdstuk 5*** werd de (diagnostische) waarde van deze adviezen voor het eerst op wetenschappelijke wijze bevestigd. Dit onderzoek liet zien dat informatie uit de anamnese de aanwezigheid van voedselallergie goed kon voorspellen, met name als het ging om

plantaardige voedingsmiddelen. Deze voorspelling bleek bij volwassenen nog beter te zijn dan bij kinderen. Orale allergie klachten (jeuk of branderigheid in de mond en/of keel) veroorzaakt door het voedingsmiddel en het hebben van allergische rhinitis (bijvoorbeeld hooikoorts) bleken bijzonder sterke voorspellers voor aanwezigheid van voedselallergie. Maagdarmklachten (overgeven of diarree) veroorzaakt door het voedingsmiddel maakten een voedselallergie minder waarschijnlijk. Op basis van onze bevindingen zou bijna de helft van de volwassenen die zich presenteren met voedingsgerelateerde symptomen correct geduid worden als niet-allergisch met alleen informatie uit de anamnese. Wel is het van belang te beseffen dat de meeste patiënten uit landen met veel berkenpollenallergie kwamen, waardoor de resultaten vooral in dergelijke landen van toepassing kunnen zijn.

Na de anamnese is het vaststellen van sensibilisatie een cruciale vervolgstap in de diagnostiek naar voedselallergie. Sensibilisatie kan worden aangetoond door huidpriktesten of bloedtesten. De bloedtesten meten de hoeveelheid IgE-antistoffen tegen het hele voedingsmiddel (extract) of tegen specifieke allergene eiwitten uit het voedingsmiddel (componenten). De laatstgenoemde testen staan bekend als 'component-resolved diagnostics' (CRD) en worden steeds vaker toegepast in de praktijk. Voor pinda is inmiddels bekend dat de hoogte van het IgE tegen eiwitcomponenten Ara h 2 en/of Ara h 6 bij zowel kinderen als volwassenen goed kan voorspellen of er sprake is van pinda-allergie. Voor hazelnoot hebben enkele studies aangetoond dat IgE tegen eiwitcomponenten Cor a 9 en Cor a 14 goed kan voorspellen of er hazelnootallergie is bij kinderen. Echter, in ***Hoofdstuk 6*** zagen we dat hazelnootallergie bij Nederlandse volwassenen niet kon worden voorspeld door de hoogte van het IgE tegen hazelnootextract en hazelnootcomponenten Cor a 1, 8, 9 en 14. Dit gebrek aan voorspellende waarde werd waarschijnlijk vooral veroorzaakt door het vaak voorkomen van sensibilisatie voor Cor a 1 bij Nederlandse volwassenen. Hazelnootcomponent Cor a 1 is kruisreactief met het belangrijkste berkallergeen. Sensibilisatie voor Cor a 1 leidt regelmatig, maar vaak ook niet, tot allergische klachten op hazelnoot. We concludeerden dat volwassen patiënten uit gebieden met veel berk-blootstelling voorlopig nog zijn aangewezen op provocatietesten voor het vaststellen van een hazelnootallergie.

Tot dusver tonen de meeste studies aan dat CRD bloedtesten de *ernst* van een voedselallergie niet goed voorspellen. Er zijn aanwijzingen dat de ernst van een voedselallergie beter voorspeld kan worden door informatie uit de anamnese te combineren met resultaten van huidpriktesten en bloedtesten. Dergelijke combinaties geven inzicht in de karakteristieken van patiënten met juist een milde of een ernstige allergie. Een recent onderzoek bestudeerde gegevens van kinderen en volwassenen die deelnamen aan het EuroPrevall onderzoek en de polikliniek allergologie bezochten met verdenking op hazelnootallergie. Deze studie liet zien dat een model waarin informatie over eczeem, hooikoorts, IgE tegen walnootextract

en IgE tegen hazelnootcomponent Cor a 14 werden samengevoegd, een goede voorspelling gaf van de ernst van hazelnootallergie.

In ***Hoofdstuk 7 en 8*** onderzochten we met dezelfde poliklinische data of dergelijke combinatiemodellen ook gebruikt kunnen worden voor het voorspellen van de ernst van walnoot- en pinda-allergie. Allereerst keken we naar de voorspellende waarde van de klinische achtergrond van de patiënt, die bestond uit informatie verkregen uit anamnese, zoals de leeftijd waarop klachten ontstonden of de aanwezigheid van een pollenallergie. Het bleek dat het hebben van een berkenpollenallergie was geassocieerd met een milde walnoot- of pinda-allergie. Kenmerken uit de klinische achtergrond die verband hielden met een ernstige walnoot- of pinda-allergie waren onder andere een familiaire allergische aanleg, het hebben van eczeem of het ontstaan van klachten bij huidcontact met het voedingsmiddel. Er waren ook verschillen tussen voorspellers voor de ernst van walnoot- en pinda-allergie. Zo voorspelde het hebben van een allergie voor bijvoet (onkruid) een ernstige walnootallergie, en het hebben van een allergie voor huisstofmijt een ernstige pinda-allergie. Bij hazelnoot (in een eerdere EuroPrevall studie), walnoot en pinda werd gezien dat kenmerken uit de klinische achtergrond waardevoller waren voor het onderscheid maken tussen een milde en ernstige allergie dan aanvullende testen voor sensibilisatie (huidpriktesten en bloedtesten). Wel bleek dat CRD testuitslagen, bovenop de klinische achtergrond, bijdroegen aan een beter onderscheid tussen milde en ernstige hazelnoot- en walnootallergie. Voor pinda-allergie was dit niet het geval. Opvallend was dat de meeste variabelen die voorspellend bleken te zijn voor een milde allergie voor hazelnoot, walnoot of pinda, wezen op een kruisreactie met pollen. Omdat pollen-gerelateerde allergie veel voorkomt in Europa en minder klinisch relevant is voor pinda-allergie dan voor notenallergie, kan dit verklaren waarom CRD meer toevoegt aan de voorspelling van de ernst van notenallergie. We concludeerden uit ***Hoofdstuk 7 en 8*** dat de klinische achtergrond van een patiënt meer bijdraagt aan de voorspelling van de ernst van noten- en pinda-allergie dan uitslagen van individuele huidpriktesten en bloedtesten, maar dat modellen waarin klinische achtergrond wordt gecombineerd met maten van sensibilisatie wel een nog betere voorspelling geven van de ernst van notenallergie. Dergelijke modellen kunnen in de toekomst ondersteuning bieden bij besluitvorming in de klinische praktijk, waardoor er minder provocatietesten nodig zullen zijn.

De bevindingen in ***Hoofdstuk 2 t/m 8*** van dit proefschrift bekrachtigen het vele voorkomen en de klinische relevantie van (met name berk) pollen-gerelateerde voedselallergie in Europa. Zoals eerder genoemd, presenteert pollen-gerelateerde voedselallergie zich meestal met milde (orale allergie) klachten. De eiwitten die hiervoor verantwoordelijk zijn worden grotendeels afgebroken door verhitting of vertering. Daarom is bij dit type voedselallergie strikte vermijding niet altijd nodig, maar passen andere (dieet)adviezen of behandelingen beter. De literatuurstudie in

Hoofdstuk 9 werd gewijd aan dit onderwerp. Verhitting van het verdachte voedingsmiddel, consumptie van hypoallergene varianten, of orale immunotherapie met het oorzakelijke voedingsmiddel, bleek bij te kunnen dragen aan de preventie of vermindering van allergische reacties bij sommige patiënten. Patiënten met een milde klachtenpresentatie in het kader van pollen-gerelateerde voedselallergie zouden daarom aangemoedigd moeten worden om hun persoonlijke dieetmogelijkheden te verkennen.

Toekomstperspectieven

Tot slot plaatsten we in ***Hoofdstuk 10*** de belangrijkste bevindingen van dit proefschrift in de context van de bestaande literatuur, waaruit ook aanbevelingen voor toekomstig onderzoek voortkwamen.

Studies die zich richten op het achterhalen van de oorzaak van voedselallergie zijn en blijven een grote prioriteit. Prospectieve projecten waarin personen gevolgd worden tot aan het ontwikkelen van allergie, kunnen veel informatie verschaffen over causaliteit. Cruciale informatie over risicofactoren zou kunnen worden verkregen door de deelnemers van de Europese EuroPrevall en Australische HealthNuts geboorte- en kindercohorten opnieuw in kaart brengen op volwassen leeftijd. Dit zou onder andere de kennis over het ontstaan of verdwijnen van voedselsensibilisatie en voedselallergie op latere leeftijd kunnen vergroten.

Uit dit proefschrift komt naar voren dat diagnostiek naar voedselallergie baat zou kunnen hebben bij verdere verkenning van voorspelmodellen waarin klinische achtergrondkenmerken met mate van sensibilisatie worden gecombineerd. Om tot de meest bruikbare modellen te komen, dienen toekomstige onderzoeksprojecten hun studiepopulatie en domein streng te definiëren aan de hand van het verdachte voedingsmiddel (bijv. noten *vs* pinda), leeftijdsgroep (bijv. zuigelingen *vs* kinderen van schoolleeftijd *vs* volwassenen) en setting (bijv. algemene populatie *vs* poliklinische populatie). Idealiter zouden zowel reacties tijdens provocatietesten als spontane reacties in het dagelijks leven meegenomen worden als uitkomstmaat, omdat klachten tijdens provocatie vaak niet overeenkomen met die van een spontane allergische reactie. Verder zouden de voorspelmodellen verrijkt kunnen worden door toevoeging van nieuwe diagnostische technieken, zoals ratio's van specifiek IgE met IgG_4 (een antistof die allergische reacties kan tegenhouden), testen die kijken naar de aanwezigheid van IgE tegen specifieke epitopen (de plaatsen waar antistoffen aan het allergeen binden), of de basofiel en mestcel activatie testen (technieken die de activiteit meten van cellen die stoffen vrijmaken die allergische klachten veroorzaken). Wat betreft pollen-gerelateerde voedselallergie, zou toekomstig onderzoek zich moeten richten op immunotherapie met zowel voeding als pollen, aangezien beide de potentie hebben om voedselallergie te behandelen of voorkomen.

Chapter 12

List of abbreviations
Contributing authors
Acknowledgements
List of publications
About the author

List of abbreviations

AD	Atopic dermatitis
AR	Allergic rhinitis
AUC	Area under the curve
BAT	Basophil activation test
CCD	Cross-reactive carbohydrate determinants
CRD	Component-resolved diagnostics
CI	Confidence interval
DBPCFC	Double-blind placebo-controlled food challenge
ECRHS	European Community Respiratory Health Survey
GI	Gastrointestinal
GP	General practitioner
HDM	House dust mite
(s)IgE	(specific) Immunoglobulin E
(s)IgG	(specific) Immunoglobulin G
IS	Inhalant sensitisation
IQR	Interquartile range
FA	Food allergy
FS	Food sensitisation
HA	Hazelnut allergy
Lasso	Least absolute shrinkage and selection operator
LMW	Low-molecular weight
LTP	Lipid transfer protein
N	Number
NA	Not applicable/available
NPV	Negative predictive value
OAS	Oral allergy symptoms
OR	Odds ratio
PA	Peanut allergy
PFA	Probable food allergy
PPV	Positive predictive value
PR-10	Pathogenesis-related protein family 10
PRFA	Pollen-related food allergy
ROC	Receiver operating characteristic
SD	Standard deviation
SE	Standard error
Sens	Sensitivity
Spec	Specificity
SPT	Skin prick test

Contributing authors

Eleonora Alquati
Department of Dermatology and Allergology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands

Riccardo Asero
Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano, Italy

Barbara K. Ballmer-Weber
Allergy Unit, Department of Dermatology, University Hospital of Zürich, Zürich, Switzerland | Clinic for Dermatology and Allergology, Kantonsspital St Gallen, St Gallen, Switzerland | Faculty of Medicine, University of Zürich, Zürich, Switzerland

Laura Barreales
Clinical Epidemiology Unit, Preventive Medicine Department, Hospital Clinico San Carlos, IdISSC, Madrid, Spain

Simona Belohlavkova
Medical Faculty and Faculty Hospital Pilsen, Charles University, Pilsen, Czech Republic

Christian Bieli
Department of Paediatric Pulmonology, University Children's Hospital, Zürich, Switzerland

Frédéric de Blay
Allergy Division, Chest Disease Department, Strasbourg University Hospital, University of Strasbourg, Strasbourg, France

Peter G. J. Burney
National Heart and Lung Institute, Imperial College London, London, UK

Michael Clausen
Landspitali University Hospital, Faculty of Medicine, University of Iceland, Reykjavik, Iceland

Mareen R. Datema
Department of Experimental Immunology, Amsterdam University Medical Centres, Location AMC, Amsterdam, The Netherlands | Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Amsterdam University Medical Centre, Amsterdam, The Netherlands

Anne M. van Dijk
Department of Dietetics, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands

Ruta Dubakiene
Medical Faculty, Vilnius University, Vilnius, Lithuania

Montserrat Fernández-Rivas
Allergy Department, Hospital Clinico San Carlos, IdISSC, ARADyAL, Madrid, Spain

Cristina Fernández-Pérez
Clinical Epidemiology Unit, Preventive Medicine Department, Hospital Clinico San Carlos, IdISSC, Madrid, Spain

Philipp Fritsche
Allergy Unit, Department of Dermatology, University Hospital of Zürich, Zürich, Switzerland

David Gislason
Landspitali University Hospital, Faculty of Medicine, University of Iceland, Reykjavik, Iceland

Mariam Hakobyan
Department of Dermatology and Allergology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands

Karin Hoffmann-Sommergruber
Medical University of Vienna, Department of Pathophysiology and Allergy Research, Vienna, Austria

Monika Jedrzejczak-Czechowicz
Department of Immunology, Rheumatology and Allergy, Faculty of Medicine, Medical University of Łódz, Łódz, Poland

Laurian Jongejan
Department of Experimental Immunology, Amsterdam University Medical Centres, Location AMC, Amsterdam, The Netherlands

Hannah M. Kansen
Department of Dermatology and Allergology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands | Department of Paedriatric

Pulmonology and Allergology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht University, Utrecht, the Netherlands

Edward F. Knol
Department of Dermatology and Allergology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands | Centre of Translational Immunology, University Medical Centre Utrecht, Utrecht, the Netherlands

André C. Knulst
Department of Dermatology and Allergology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands

Marek L. Kowalski
Department of Immunology, Rheumatology and Allergy, Faculty of Medicine, Medical University of Łódz, Łódz, Poland

Tanya Z. Kralimarkova
Clinical Centre of Allergology, Medical University of Sofia, Sofia, Bulgaria

Ischa Kummeling
Department of Dermatology and Allergology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands | National Heart and Lung Institute, Imperial College London, London, UK

Thuy-My Le
Department of Dermatology and Allergology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands

Ans F. M. Lebens
Department of Dermatology and Allergology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands

Jonas Lidholm
Thermo Fisher Scientific, Uppsala, Sweden

E. N. Clare Mills
Division of Infection, Immunity and Respiratory Medicine, Manchester Institute of Biotechnology & Lydia Becker Institute of Immunology and Inflammation, University of Manchester, Manchester, United Kingdom

Tihomir B. Mustakov
Clinical Centre of Allergology of the Alexandrovska Hospital, Medical University of Sofia, Sofia, Bulgaria

Harmieke van Os-Medendorp
Department of Dermatology and Allergology, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands | Saxion University of Applied Sciences, Deventer, The Netherlands

Henny G. Otten
Centre of Translational Immunology, University Medical Centre Utrecht, Utrecht, the Netherlands

Nikolaos G. Papadopoulos
Division of Infection, Immunity & Respiratory Medicine, Manchester Institute of Biotechnology, University of Manchester, Manchester, United Kingdom | Allergy Department, 2nd Paedriatric Clinic, University of Athens, Athens, Greece

Bo Pontoppidan
Thermo Fisher Scientific, Uppsala, Sweden

Todor A. Popov
University Hospital Sv. Ivan Rilski, Sofia, Bulgaria

James Potts
National Heart and Lung Institute, Imperial College London, London, UK

Nayade del Prado
Clinical Epidemiology Unit, Preventive Medicine Department, Hospital Clinico San Carlos, IdISSC, Madrid, Spain

Ashok Purohit
Allergy Division, Chest Disease Department, Strasbourg University Hospital, Strasbourg, France

Ronald van Ree
Department of Experimental Immunology, Amsterdam University Medical Centres, Location AMC, Amsterdam, The Netherlands | Department of Otorhinolaryngology, Amsterdam University Medical Centres, Location AMC, Amsterdam, The Netherlands

Isabel Reig
Allergy Department, Hospital Clinico San Carlos, IdISSC, Madrid, Spain

Alexandros Sakellariou
Allergy Department, 2nd Paedriatric Clinic, University of Athens, Athens, Greece

Suranjith L. Seneviratne
Institute of Immunity and Transplantation, University College London, London, United Kingdom

Athanassios Sinaniotis
Allergy Department, 2nd Paedriatric Clinic, University of Athens, Athens, Greece

Emilia Vassilopoulou
Department of Nutritional Sciences and Dietetics, International Hellenic University, Thessaloniki, Greece

Serge A. Versteeg
Department of Experimental Immunology, Amsterdam University Medical Centres, location AMC, Amsterdam, The Netherlands

Stefan Vieths
Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, Langen, Germany

Paco M. J. Welsing
Division of Internal Medicine and Dermatology, University Medical Centre Utrecht, Utrecht University, The Netherlands

Paraskevi Xepapadaki
Allergy Department, 2nd Paedriatic Clinic, University of Athens, Athens, Greece

Aeilko H. Zwinderman
Department of Clinical Epidemiology, Amsterdam University Medical Centres, location AMC, Amsterdam, The Netherlands

Acknowledgements (Dankwoord)

Het boekje is er! Met trots en waardering kijk ik terug op de afgelopen vier jaren en sta ik stil bij alle hulp en steun die ik heb gekregen om tot dit eindresultaat te komen. Aan alle betrokkenen; bedankt, thank you, Þakka þér fyrir, ačiū, dziękuję, děkuji, merci, danke, gracias, grazie, Благодаря, gracias, ευχαριστώ, go raibh maith agat!

Patiënten en participanten, zonder jullie was dit proefschrift er met geen mogelijkheid gekomen. Bedankt voor jullie bereidheid en alom onmisbare bijdrage aan dit onderzoek.

André, mooi hoe jij altijd de muziek in de (voedsel)allergie weet over de dragen aan anderen. Ik heb enorm veel gehad aan jouw enthousiasme, kritische blik, vermogen om mee te denken, en de klinische kennis die je mij, samen met Thuy-My, hebt bijgebracht op de poli. De prioriteit die je aan mijn stukken gaf tijdens de welbekende 'eindsprint' waardeer ik enorm. Vanuit jouw vertrouwde helikopter wist jij de onderzoeksprojecten in dit proefschrift tot een hoger niveau te tillen.

Ronald, wat een geniale toevoeging aan mijn promotieteam. Met jou als echte EuroPrevall-kenner en pro op gebied van de moleculaire allergologie, was er geen berg te hoog. Je was ook altijd bereid om 'even' naar Utrecht te komen voor een overleg. Bedankt voor het delen van jouw expertise en netwerk, het vertrouwen in mijn capaciteiten en de aandacht voor het leven buiten het onderzoek. Ik hoop dat er meer mooie samenwerkingen tussen de teams in Amsterdam en Utrecht volgen!

Thuy-My, over echte EuroPrevall-kenners gesproken… Prachtig dat jij tijdens jouw promotie de Nederlandse tak van het EuroPrevall project hebt getrokken en mij vervolgens de afgelopen vier jaren hebt begeleid om de data uit heel Europa om te toveren tot de vele mooie artikelen in dit boekje. Ik sta altijd weer versteld van jouw optimisme, efficiëntie, creatieve oplossingen en het ongekende niveau waarop jij functioneert op slechts drie uur slaap. Je bent een fantastische copromotor. Je inkortsuggesties waren onmisbaar, je sinterklaasgedichten onvergetelijk.

Paco, jouw statistische en methodologische inzichten hebben de kwaliteit van de artikelen in dit boekje waanzinnig versterkt. Ik ben ontzettend blij dat jij je twee jaar geleden als copromotor bij ons team besloot de voegen. Bedankt voor de nuttige sparmomenten, je geduldige uitleg, en je juist niet-klinische, maar epidemiologische blik (ook al heb je je inmiddels ontwikkeld tot een ware amateur-allergoloog). Ik kan nog veel leren van hoe jij altijd in mogelijkheden denkt.

Harmieke, als een van de oorspronkelijke copromotoren heb jij de eerste twee jaren een sleutelrol gespeeld bij de vormgeving van mijn promotietraject en dit boekje.

Fijn hoe jij altijd bereid was om je klinische en epidemiologische kennis te delen, mij steunde in mijn keuze voor (en enthousiasme over) de master Epidemiologie, en altijd ook even checkte hoe het nou écht met me ging. Jouw nieuwe collega's zijn vast ook heel blij met je!

Leden van de beoordelings- en promotiecommissie, prof. dr. C.K. Van der Ent, prof. dr. J. Garssen, prof. dr. G.F. Houben, prof. dr. J.O. Hourihane, prof. dr. L. Meyaard, prof. dr. E.N.C. Mills, prof. dr. K.G.M. Moons, hartelijk dank voor de tijd die jullie hebben genomen om dit proefschrift te beoordelen en/of te opponeren tijdens mijn verdediging. Thank you for taking the time to assess this thesis and/or for participating as an opponent in my PhD defence.

A huge thank you to the entire **EuroPrevall team**, who made the majority of the papers in this thesis possible. Your hard work and immense contributions are greatly appreciated. I would especially like to acknowledge the excellent coordination of the EuroPrevall projects by and experienced insights from Peter Burney, Ischa Kummeling, Clare Mills, Montserrat Fernández-Rivas and Barbara Ballmer-Weber.

Alle co-auteurs wil ik bedanken voor hun waardevolle bijdragen aan dit proefschrift. Naast mijn begeleiders en het hele EuroPrevall team, ook Mareen, Hannah, Anne, Edward, Henny, Eleonora en Mariam, bedankt voor de fijne samenwerking.

Afdeling dermatologie en allergologie, wat een geweldige werkplek. Ik kijk ernaar uit om terug te keren als AIOS. Er was altijd veel gezelligheid tijdens de werkoverleggen, koffiemomentjes en lunchpauzes. Jantine en Miranda, bedankt voor al jullie uitzoek- en meedenkwerk, ondanks dat er genoeg op jullie bordje lag. Polikliniek allergologie, bedankt voor de laagdrempelige benaderbaarheid en voor het creëren van een fijne werk- en leeromgeving. Anne, Andrea, Anouska, Maartje, Petra, de voedselallergie nabesprekingen waren altijd goed met jullie erbij! Ans, Jos, Annemieke, Ilona, bedankt voor de fijne samenwerking en de gezelligheid. Marieke, bedankt voor je hulp bij en de overname van de datamanager rol voor de acute ontsteking. Afdeling kinderallergologie in het WKZ, Kors, Yolanda, Eva, Ted, Francine, Hannah en Maaike, fijn dat jullie bereid waren om af en toe jullie expertise op mijn bevindingen los te laten.

Mede-promovendi en G02-kamergenoten, Daphne, Lieneke, Mark S, Mehran, Anna, Fleur, Lotte, Roselie, Celeste, Michelle, Hannah K, Maaike, Astrid, Hannah F, Mark B, Judith, Jorien, Floor, Henrike, Mignon, Mary-Ann – zonder jullie hadden die vier jaren er heel anders uitgezien. Ik heb genoten van de goede samenwerking, de geweldige congressen, de gezelligheid op de kamer en de vele mooie borrels. Dat belooft veel goeds voor de toekomst!

Acute ontsteking werkgroep, André, Thuy-My, Heike, Geert, Henny, Edward, Kitty, Stans, Astrid, Maartje, Anna, Mark en Mehran, we hebben samen een hoop goede en gezellige overlegmomenten gehad op de dinsdagmiddag. Bedankt voor jullie vele nuttige inzichten. Heike, jij liet mij als student kennismaken met onderzoek bij de allergologie in het UMC Utrecht. Bedankt voor de inspiratie en je vertrouwen.

Studenten, Eleonora, Amber en Mariam, wat een fijne en gezellige samenwerkingen. Bedankt voor jullie inzet!

Anne Meike en Hannah, jullie zijn het ideale paranimf-duo. Met jullie aan mijn zijde is een mooie verdediging gegarandeerd. AM, we hebben samen al zoveel avonturen beleefd. Genieten dat deze er nu bij komt, als één van de vele legendarische momenten die 2021 op de planning heeft staan. Han, van allergie-collega tot epi-buddy tot lieve vriendin, geweldig leuk om de rollen kort na jouw verdediging om te mogen draaien.

Lieve **vrienden en vriendinnen**, jullie zorgden ervoor dat de mooie momenten altijd overheersten. Madelein, Reinoud, Anne Meike, Frits, Mieke, Martin, Hanna, Koen, Matthias, Martina, Annelienke, Donald, Hannah, Nienke, Peter, Djurre en Casper, jullie hebben in het bijzonder voor de nodige afleiding gezorgd. Frits, dankjewel voor je lieve hulp en Illustrator skills bij het maken van de kaft, en Hanna, jij voor de push die ik nodig had om de kaft zelf te ontwerpen.

Ingrid en Elsa, bedankt voor het warme welkom in jullie gezin en de oprechte belangstelling voor mijn promotietraject. Jullie 'gewoon proberen' mentaliteit, levendige discussies en de liefdevolle en persoonlijke sinterklaasavonden hebben mijn leven een stukje mooier gemaakt.

Mum, Dad, Brendan, Maura, and Emma, thank you for setting great examples and for your unconditional love, support and pride. Maurs and Ems, I really appreciate your patient spell checks and useful input. Thank you for encouraging me to ask for help, despite your own full schedules. Mum, thanks for always being there, whatever the reason.

Sjaak, er is niemand zoals jij. Je bent ongekend positief, hebt lol in het leven, en weet precies wat je moet doen om mij weer een nieuwe energie-boost te geven. Ik kan niet wachten om met je te trouwen, maar nu eerst uitkijken naar het best mogelijke reisavontuur!

List of publications

This thesis, published

Lyons SA, Dijk AMV, Knulst AC, Alquati E, Le TM, Os-Medendorp HV. Dietary interventions in pollen-related food allergy. Nutrients. 2018;10(10):1520.

Lyons SA, Burney PGJ, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, Clausen M, et al. Food allergy in adults: substantial variation in prevalence and causative foods across Europe. J Allergy Clin Immunol Pract. 2019;7(6):1920-8 e11.

Lyons SA, Clausen M, Knulst AC, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, et al. Prevalence of food sensitization and food allergy in children across Europe. J Allergy Clin Immunol Pract. 2020;8(8):2736-46 e9.

Lyons SA, Knulst AC, Burney PGJ, Fernandez-Rivas M, Ballmer-Weber BK, Barreales L, et al. Predictors of food sensitization in children and adults across Europe. J Allergy Clin Immunol Pract. 2020;8(9):3074-83 e32.

Lyons SA, Knulst AC, Burney PGJ, Fernandez-Rivas M, Ballmer-Weber BK, Barreales L, et al. Predicting food allergy: the value of patient history reinforced. Allergy. 2020. In press.

Lyons SA, Datema MR, Le TM, Asero R, Barreales L, Belohlavkova S, et al. Walnut allergy across Europe: distribution of allergen sensitization patterns and prediction of severity. J Allergy Clin Immunol Pract. 2020; S2213-2198(20)30935-1. In press.

This thesis, submitted

Lyons SA, Datema MR, Fernandez-Rivas MR, Ballmer-Weber B, Knulst AC, Asero R, et al. Estimating the risk of severe peanut allergy using clinical background and IgE sensitisation patterns. Submitted.

Lyons SA, Welsing PMJ, Hakobyan M, Kansen HM, Knol EF, Otten HG, et al. IgE to hazelnut allergen components does not predict hazelnut challenge outcome in Dutch adults. Submitted.

Other publications

Lyons SA, Su T, Vissers LET, Peters JPM, Smit AL, Grolman W. Fascia compared to one-piece composite cartilage-perichondrium grafting for tympanoplasty. Laryngoscope. 2016;126(7):1662-70.

Kansen HM, van Erp FC, Knulst AC, Ehlers AM, **Lyons SA,** Knol EF, et al. Accurate prediction of peanut Allergy in one-third of adults using a validated Ara h 2 cutoff. J Allergy Clin Immunol Pract. 2020;S2213-2198(20)31245-9. In press.

About the author

Sarah was born on December 30th 1990 in Cork, Ireland. She attended secondary school at the Gertrudiscollege in Roosendaal, the Netherlands, from which she obtained her VWO gymnasium diploma *cum laude* in 2008. Her medical training at Utrecht University commenced that same year. During her degree, she explored the various fields of medicine extensively, particularly enjoying both curricular and extra-curricular electives in dermatology, internal medicine and infectious diseases across the world. Sarah's affinity with allergy research became apparent during her final-year research internship on drug allergy in the department of dermatology and allergology at the University Medical Centre of Utrecht (UMCU) under the supervision of dr. Heike Röckmann. After obtaining her medical degree in 2015, she first gained a year of hands-on clinical experience as a junior doctor in internal medicine at the St. Antonius Hospital in Utrecht. She then returned to the UMCU's department of dermatology and allergology to start her PhD trajectory on food allergy under the supervision of prof. dr. André Knulst, prof. dr. Ronald van Ree, dr. Thuy-My Le, dr. Paco Welsing and dr. Harmieke van Os-Medendorp, which resulted in the present thesis. As part of her PhD training, Sarah also successfully completed a postgraduate master's degree in epidemiology at Utrecht University's Graduate School of Life Sciences in 2019. Sarah is excited to be starting the dermatology residency programme at the UMCU in 2022, after first spending several months globetrotting with her fiancé Sjaak, COVID-19 allowing.