Exploring the prevalence, natural history and adverse events related to tree nut allergy

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Abstract

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Peanut and tree nut allergies are the most commonly reported trigger of food induced anaphylaxis and fatalities. Whilst most childhood allergies are outgrown by school age, peanut and tree nut allergy resolution has been reported to be as low as 10%. Despite the potential severity and lifelong nature of both peanut and tree nut allergy much of the literature to date has focused on peanut allergy epidemiology, and tree nut allergy prevalence, natural history while adverse events have been under studied. There is very limited data on the prevalence of allergies to the individual tree nuts nor the factors related to development of tree nut allergy.

Therefore, this thesis explores several key areas regarding tree nut allergy including the prevalence, elements of the natural history and development of tree nut allergy, as well as the frequency of adverse food reactions to tree nuts. This PhD has utilised data from the population-based HealthNuts and SchoolNuts studies and the Royal Children's Hospital allergy clinic populations all based in Melbourne, Australia.

In a systematic review I published, I found limited tree nut allergy prevalence estimates based on challenge-confirmed outcomes with current estimates less than 2%, while probable tree nut allergy prevalence ranged from 0.05 to 4.9%. Prevalence of individual tree nut allergies varied significantly by region with hazelnut the most common tree nut allergy in Europe, walnut and cashew in the USA and Brazil nut, almond and walnut most commonly reported in the UK. There was no challenge-confirmed Australian tree nut allergy data.

Among 6 year old children in the HealthNuts study, 3.3% had challenge confirmed tree nut allergy and 2.6% of 10 to 14 year olds self-reported one or more tree nut allergies in the SchoolNuts study. Cashew was the most common tree nut allergy both at 6 and 10 to 14 years of age.

Among those with peanut or egg allergy at 12 months of age in the HealthNuts study, sensitisation rates to tree nut were as high as 48%, with 39% of those tree nut sensitised at 12 months tree nut allergic at 6 years of age.

Confirming other reports of adverse food reactions, the work conducted as part of this PhD has found peanut and tree nut the most common triggers of adverse food reactions in the past year for children aged 10 to 14 years, with cashew the most common individual tree nut trigger reported.

Finally, a cashew SPT wheal size of 10mm was found to have 95% PPV to challenge confirmed cashew allergy using the population based HealthNuts and SchoolNuts cohorts.

In summary, the results presented in this thesis have reported the first challenge confirmed tree nut allergy prevalence rates in Australia and have highlighted one of the highest reported tree nut allergy rates in the world to date, with cashew the most common tree nut allergy. With up to half of those with food allergies already sensitised to tree nuts as early as one year of age, improved methods for identifying and targeting children at highest risk of tree nut allergy along with development of early prevention strategies are desperately needed, with cashew allergy a priority tree nut in Australia.

Declaration

This is to certify that:

i. This thesis comprises only my original work towards the Doctor of Philosophy and includes nothing, which is the outcome of work done in collaboration except where specifically indicated in the text.

ii. This thesis has not been previously submitted, in part or whole, to any university of institution for any degree, diploma, or other qualification.

iii. The thesis is less than 100,000 words in length, exclusive of tables, figures, bibliographies and appendices.

Signed:

Date:

Preface

This thesis is based primarily on data collected by the HealthNuts and SchoolNuts studies. The HealthNuts study was developed and conducted by Professor Katie Allen (Principal Investigator) in collaboration with Professor Shyamali Dharmage, Associate Professor Lyle Gurrin, Dr. Nicholas Osborne, Professor Melissa Wake, Professor Mimi Tang, Professor Anne-Louise Ponsonby, Dr Melanie Matheson, Dr Adrian Lowe and Dr David Hill. Waves 1, 2 and 3 of the HealthNuts study, including recruitment, clinical assessment and some data cleaning was completed by the HealthNuts study team and past PhD students prior to the commencement of this PhD. I contributed to data entry and data cleaning of wave 3 data as well as all of the data analysis that was included in this thesis.

The SchoolNuts study was also developed and conducted by Professor Katie Allen (Principal Investigator) in collaboration with Professor Shyamali Dharmage, Associate Professor Lyle Gurrin, Professor Susan Sawyer, Professor George Patton, Professor Jo Douglass, Associate Professor Peter Vuillermin and Dr Jennifer Koplin. I participated in some of the recruitment school visits, data entry and cleaning and the data analysis included in this thesis.

This thesis contains four publications of which I am primary author, conducted the data analysis, wrote the first draft of the manuscript, responded to peer review and contributed more than 50% of the work.

The systematic review of tree nut allergy prevalence, presented in Chapter 4, was planned together with Katie Allen, Shyamali Dharmage, and Jennifer Koplin. Caroline Lodge and Shyamali Dharmage provided methodological and statistical support for the analysis of the data. All named co-authors contributed to interpretation of the results and provided intellectual input on drafts of the manuscript and response to peer review.

The publication on self-reported adverse food reactions and anaphylaxis, presented in Chapter 5, was planned together with Katie Allen, Jennifer Koplin and the SchoolNuts Investigators. Shyamali Dharmage and Jennifer Koplin provided statistical support for the analysis of the data. All named co-authors contributed to interpretation of the results and provided intellectual input on drafts of the manuscript and response to peer review.

The publication on the prevalence and development of tree nut allergy, presented in Chapter 6, was planned together with Katie Allen and Jennifer Koplin. Jennifer Koplin, Shyamali Dharmage and Ann-Louise Ponsonby provided statistical support for the analysis of the data. All named co-authors contributed to interpretation of the results and provided intellectual input on drafts of the manuscript and response to peer review.

The final publication on cashew oral food challenge outcomes and positive predictive values for cashew SPT, presented in Chapter 7, was planned together with Katie Allen, Jennifer Koplin and Rachel Peters. Data from HealthNuts and SchoolNuts was combined with data from two allergy clinics -The Royal Children's Hospital and Melbourne Allergy and Children's Centre (MACCS). Use of the RCH data required an ethics application which was prepared by myself with input from all named authors. All RCH and MACCS data was collected and analysed by myself. Lyle Gurrin and Rachel Peters provided statistical support for the analysis of the data. All named co-authors contributed to interpretation of the results and provided intellectual input on drafts of the manuscript which has recently been submitted for publication.

My PhD was funded by a scholarship from the Centre for Food and Allergy Research (CFAR) a National Health and Medical Research funded Centre for Excellence. I also received a Royal Children's Hospital travelling scholarship to present my research at an international conference.

Over the course of my PhD I have been involved in ten additional papers as a co-author. I have also participated in two Centre for Food and Allergy Research Summits and been involved in the write up and publication of proceedings. Details of all co-authored manuscripts published over the course of my PhD are listed under the other co-authored publications in the Publications and Presentations section. Finally, I acted as a peer reviewer for several journals including Clinical and Experimental Allergy, Paediatric Allergy and Immunology and the Journal of Nutrition and Dietetics.

Acknowledgements

My PhD journey has been a long one intertwined with my ongoing clinical work as a food allergy dietitian and all the personal roles we collect along the way (wife, Mum, friend, sister, daughter etc, etc). I have been privileged to have an extremely experienced group of PhD supervisors and I would like to take this opportunity to thank, Professor Katie Allen, Dr Jennifer Koplin, Professor Mimi Tang, Professor Shyamali Dharmage and Dr Kirsten Perrett for their guidance and support over the past 5 years. Their collective experience in food allergy, epidemiology, statistics and conducting and publishing research has provided me with an amazing foundation on many aspects of research. I would particularly like to thank my primary supervisor Katie as without her initial support and encouragement that I could work a PhD into my life this would not have started. She is one inspirational person with extensive expertise and an enviable drive and passion for clinical allergy and research. I would also like to thank Jennifer Koplin who has walked me through all aspects of statistical analysis and scientific writing her advice, guidance and patience are an asset to the research group and were invaluable throughout my PhD. I would like to thank Shyamali for the scientific rigour she has taught me regarding the application of epidemiological concepts and publishing research and Mimi for her clinical and research expertise. I would also like to thank Harriet Hiscock for her guidance and support as Advisory panel chair and my colleague and fellow dietitian Heather Gilbertson as an advisory panel member.

I would like to thank the study participants and families of the HealthNuts and SchoolNuts studies for their time and participation and the various funding bodies that made these studies possible: the Australian National Health and Medical Research Council, the Ilhan Food Allergy Foundation, the Centre for Food and Allergy Research, The University of Melbourne and the Murdoch Children's Research Institute. I would also like to formally acknowledge and thank the NHMRC funded Centre for Food and Allergy research for my PhD scholarship funding and scientific oversight of my PhD.

I also thank the Gastro and Food Allergy Research group members at the Murdoch Children's Research Institute not only for your commitment and dedication to your work but for embracing and supporting me as a PhD student within your group.

Similarly my colleagues in the departments of Allergy & Immunology and Nutrition & Food Services at the Royal Children's Hospital who have either directly participated in my research, co-authored papers or just kept checking in on how things were tracking.

Finally and most significantly I would also like to thank my family, my husband Steve and my children Tom and April. I know it has been hard when I have been working long hours on something or was away at conferences but your patience and love has been noted and so appreciated. I am blessed with a great family and awesome friends who may not have got this but they have come along for the ride and supported me all the way.

Summary of Publications and Presentations

First author publications

1. **McWilliam VL**, Peters R, Tang MLK, Dharmage SC, Ponsonby AL, Gurrin L, Perrett Koplin J, Allen KJ. **Patterns of tree nut sensitisation and allergy in the first 6 years of life in a population-based cohort**. Journal of Allergy and Clinical Immunology. Article in press, July 2018. **Chapter 6.**
2. **McWilliam VL**, Koplin JJ, Field MJ, Sasaki M, Dharmage SC, Tang MLK, Sawyer SM, Peters RL, Allen KJ. **Self-reported adverse food reactions and anaphylaxis in the SchoolNuts study**. Journal of Allergy and Clinical Immunology. 141:3, March 2018. **Chapter 5.**
3. **McWilliam VL**, Koplin J, Lodge C, Tang M, Dharmage SC, Allen KJ. **The Prevalence of Tree Nut Allergy: A Systematic Review**. Current Allergy & Asthma Reports 15:54, 2015. **Chapter 4.**
4. **McWilliam, VL,** Heine R, Tang MLK, Allen KJ. **Multiple food protein intolerance of infancy or severe spectrum of non-IgE-mediated cow's milk allergy?—A case series**. Journal of Allergy and Immunology-In Practice, Mar/April 2016.

Publications currently under review

McWilliam VL, Peters RL ,Allen KJ, Dharmage S, Ponsonby AL,Tang MLK, Smart J, Perrett K, D Tey D, Robinson M, Taranto M, Koplin J, and Gurrin L. **SPT predictive values for the outcome of cashew challenges in children.** Submitted to the Journal of Allergy and Clinical Immunology, October 2018

Other co-authored papers during the course of this PhD

1. Sandra Vale, Merryn J Netting, Lara S Ford, Briony Tyquin**, Vicki McWilliam**, Dianne E Campbell. **Anaphylaxis Management in Australian Schools: Review of Guidelines and Adrenaline Auto injector Use** Journal of Paediatric Child Health, October 2018, in press.
   I provided intellectual input into the drafting and revising of the manuscript.
2. Sasaki M, Peters RL, Koplin JJ, Field MJ, **McWilliam V**, Sawyer SM, Vuillermin PJ, Pezic A, Gurrin LC, Douglass JA, Tang MLK, Dharmage SC, Allen KJ. **Risk factors for food allergy**

in early adolescence: The SchoolNuts study. J Allergy Clin Immunol Pract.2018 Mar - Apr;6(2):496-505.

Data cleaning and analysis work undertaken as part of my PhD work on the *SchoolNuts study was included in this manuscript. I also provided intellectual input into the drafting and revising of the manuscript.*

3. Rachel L. Peters, Koplin, Dharmage, Mimi, McWilliam, Lyle, Melanie Neeland, Adrian J. Lowe, Anne-Louise Ponsonby, Katrina J. Allen, **Early exposure to cow's milk protein is associated with a reduced risk of cow's milk allergic outcomes**

   I provided intellectual input into the drafting and revising of the manuscript.

4. Marnie Robinson, Jennifer J. Koplin, Michael J. Field, Mari Sasaki, Rachel L. Peters**, Vicki McWilliam**, Susan M. Sawyer, MD, George C. Patton, Peter J. Vuillermin, Jo Douglass, Lyle C. Gurrin, Mimi L.K. Tang, PhD, Shyamali C. Dharmage, Katrina J. Allen,. **Patterns of carriage of prescribed adrenaline auto injectors in 10 to 14 year old food-allergic students: A population-based study.** J Allergy Clin Immunol Pract.2018 Jul 19. pii: S2213-2198(18).

   Data cleaning and analysis work undertaken as part of my PhD work on the SchoolNuts study was included in this manuscript. I also provided intellectual input into the drafting and revising of the manuscript.

5. Vermeulen EM, Koplin JJ, Dharmage SC, Gurrin LC, Peters RL, **McWilliam VL**, Ponsonby AL, Dwyer T, Lowe AJ, Tang MLK, Allen KJ**. Food allergy is an important risk factor for childhood asthma, irrespective of whether it resolves**. Journal of Allergy and Clinical Immunology - In Practice. In Press, June 2018

   I provided intellectual input into the drafting and revising of the manuscript.

6. Sasaki M, Koplin JJ, Dharmage SC, Field MJ, Sawyer SM, **McWilliam VL**, Peters RL, Gurrin LC, Vuillermin PJ, Douglass J, Pezic A, Brewerton M, Tang MLK, Patton GC, Allen KJ. **Prevalence of clinic-defined food allergy in early adolescence: The SchoolNuts Study**. Journal of Allergy and Clinical Immunology. 141 (1) January, 2018.

   Data cleaning and analysis work undertaken as part of my PhD work on the *SchoolNuts study was included in this manuscript. I also provided intellectual input into the drafting and revising of the manuscript.*

7. Netting MJ, Campbell DE, Koplin JJ, Beck KM, **McWilliam VL**, Dharmage SC, Tang MLK, Ponsonby AL, Prescott SL, Vale S, Loh RKS, Makrides M, Allen KJ, Centre for Food and Allergy Research, the Australasian Society of Clinical Immunology and Allergy, the

National Allergy Strategy and the Australian Infant Feeding Summit Consensus Group. **An Australian consensus on infant feeding guidelines to prevent food allergy: outcomes for the Australian infant feeding summit**. The Journal of Allergy and Clinical Immunology: In Practice. 5 (6), Nov-Dec 2017.

I attended the roundtable day as a stakeholder and recorded notes of the proceedings that formed the background for the manuscript. I also provided intellectual input into the drafting and revising of the manuscript.

8. Loke P, Heine R, **McWilliam VL**, Cameron DJS, Tang MLK, Allen KJ. **Fecal microbial transplantation in a pediatric case of recurrent Clostridium difficile infection and specific antibody deficiency**. *Pediatric Allergy & Immunology*, 27:8, December, 2016.

   I provided intellectual input into the drafting and revising of the manuscript.

9. Zurzolo GA, Koplin JJ, Ponsonby AL, **McWilliam VL**, Dharmage S, Heine RG, Tang MLK, Prescott S, Campbell DE, Loh R, Rueter K, Netting M, Frith K, Norton W, Said M, Gold M, Lee NA, Mathai M, de Courten M, Allen KJ. **Consensus of stakeholders on precautionary allergen labelling: A report from the Centre for Food and Allergy Research**. Journal of Paediatrics and Child Health. 52:8, August 2016.

   I attended the roundtable day as a stakeholder and recorded notes of the proceedings that formed the background for the manuscript. I also provided intellectual input into the drafting and revising of the manuscript.

10. Panjari M, Koplin JJ, Dhramage SC, Peters RL, Gurrin LC, Sawyer SM, **McWilliam VL**, Eckert JK, Vicendese D, Erbas B, Matheson MC, Tang MLK, Douglass J, Ponsonby AL, Dwyer T, Goldfield S, Allen KJ. **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**. Clinical and Experimental Allergy. 46:4: April 2016.

    I provided intellectual input into the drafting and revising of the manuscript.

11. Beck C, Koplin J, Dharmage S, Wake M, Gurrin L, **McWilliam VL**, Tang MLK, Sun C, Foskey R, Allen KJ**. Persistent Food Allergy and Food Allergy Coexistent with Eczema is Associated with Reduced Growth in the First 4 Years of Life.** Journal of Allergy and Immunology -In Practice. Vol, 4:2, March /April, 2016.

    I contributed to data analysis, interpretation of the results and provided intellectual input to the drafting of the manuscript.

Presentations and posters relevant to this thesis

1. **McWilliam VL**, Koplin J, Lodge C, Tang M, Dharmage SC, Allen KJ. **The Prevalence of Tree Nut Allergy: A Systematic Review** Presented at the Food, Allergy and Nutrition Symposium hosted by the NHMRC funded Centre for Food & Allergy Research (CFAR) and the CRE in Foods for Future Australians in 2015. It was also presented as an abstract and poster at the Australian Society of Clinical Immunology and Allergy (ASCIA) annual scientific meeting in 2015 both held in Adelaide, Australia
2. **McWilliam VL**, Koplin JJ, Field MJ, Sasaki M, Dharmage SC, Tang MLK, Sawyer SM, Peters RL, Allen KJ. **Self-reported adverse food reactions and anaphylaxis in the SchoolNuts study** This research was presented at the European Academy of Allergy and Clinical Immunology (EAACI) Congress in Vienna, Austria in June 2016 as an oral presentation. It was awarded best oral presentation in the anaphylaxis section of the meeting. It was also presented at the University of Melbourne's Department Paediatrics "3 minute Thesis" competition in October 2016 and one 1st prize and the Centre for Food and Allergy Research roundtable symposium on "Food Allergy in Schools", held in Melbourne in February 2017
3. **McWilliam VL**, Peters R, Tang MLK, Dharmage SC, Ponsonby AL, Gurrin L, Perrett Koplin J, Allen KJ. **Patterns of tree nut sensitisation and allergy in the first 6 years of life in a population-based cohort**. This research was presented as an oral poster at the European Allergy and Clinical Immunology (EAACI) Paediatric Meeting (PAAM) in London October 2017, the .Centre for Food and Allergy Research Strategic Planning Day in May 2018 in Canberra and the University of Melbourne Student research symposium and was awarded 3rd prize.

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Figure 2.2 Mechanism of IgE-mediated food allergy reactions and symptoms	Renz H, Allen KJ, Sicherer SH, et al. Food allergy. *Nat Rev Dis Primers.* 2018;4:17098	Yes
Figure 2.3 Algorithm for the diagnosis of food allergy	Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *The Journal of Allergy and Clinical Immunology.* 2018;141(1):41-58	Yes
Figure 2.4 Standardised stopping criteria for oral food challenges	Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, 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. The Journal of allergy and clinical immunology. 2012;130(6):1260-1274	Yes
Figure 2.5 Environmental and lifestyle factors related to microbial exposure and their effect on the risk of developing food allergy	Aitoro R, Paparo L, Amoroso A, et al. Gut Microbiota as a Target for Preventive and Therapeutic Intervention against Food Allergy. *Nutrients.* 2017; 9(7)	Yes
Figure 3.1The HealthNuts study sample, representative of a Victorian birth cohort	Osborne et al, Clinical and Exp Allergy, 2010	Yes
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List of Abbreviations

Abbreviation	Full Text
A&AA	Allergy and Anaphylaxis Australia
ASCIA	Australasian Society of Clinical Immunology and Allergy
BAT	Basophil activation test
CFAR	Centre for Food and Allergy Research
CRD	Component resolved diagnostics
CRE	Centre for Research Excellence
DBPCFC	Double-blind, placebo-controlled food challenge
EAACI	European Academy of Allergy and Clinical Immunology
FSANZ	Food standards Australia New Zealand
ICSEA	Index of Community Socio-educational Advantage
IgE	Immunoglobulin E
LEAP	Learning Early About Peanut study
LR	Likelihood ratio
NHMRC	National Health and Medical Research Council

Abbreviation	Full Text
NIAID	National Institute of Allergy and Infectious Disease
NPV	Negative predictive value
OAS	Oral allergy syndrome
OFC	Oral food challenge
OIT	Oral immunotherapy
PAAM	Paediatric Allergy and Asthma Meeting
PFS	Pollen food syndrome
PPV	Positive predictive value
sIgE	Serum specific immunoglobulin E
SPT	Skin prick testing
UK	United Kingdom
UKFAR	United Kingdom Fatal Anaphylaxis Registry
US	United States of America
WAO	World Allergy Organisation

List of Appendices

1.INTRODUCTION

1.1 Rationale

Food allergy affects up to 10% of children and 2-3% of adults, and appears to be increasing in prevalence.[2-4] Just eight foods account for more than 80% of all childhood IgE-mediated food allergy: cow's milk, soy, egg, wheat, peanuts, tree nuts, fish and shellfish. Cow's milk and egg are reported as the most common IgE-mediated food allergies in children, however the prognosis is good with resolution expected for the majority of children by school age. Peanut and tree nut allergies on the other hand tend to be lifelong and are the cause of most food related anaphylaxis and deaths.[5]

Tree nut is the collective term used to describe an edible range of seeds and fruits that predominantly grow on trees. Tree nuts most likely to result in IgE-mediated food allergy reactions are almond, Brazil nut, cashew, hazelnut, macadamia, pecan, pistachio and walnut. Contrary to popular belief peanuts are not tree nuts and are in fact a groundnut and classified as a legume however, although botanically unrelated, tree nut and peanut allergies share many clinical similarities and are also reported to commonly co-exist. Despite the potential severity of both peanut and tree nut allergy much of the literature to date has focused on peanut allergy epidemiology while tree nut allergy prevalence, natural history and adverse events have been under reported.

There is very limited data on the prevalence of allergies to the individual tree nuts. This may be due to the fact that historically, patients diagnosed with peanut and/or tree nut allergies were advised to avoid all nuts. This stemmed from clinical assumptions regarding taxonomy, that tree nuts from similar families would have similar allergic capabilities and the high level of cross reactivity that had been established between peanuts and tree nuts [6]. The clinical dilemma is that these cross reactivity relationships do not always result in co-sensitisation on allergy testing or actual allergic reactions. Practical considerations also impacted advice to avoid all nuts. These considerations include potential difficulty for patients to distinguish one nut from another,[7,8] the potential cross contamination of nuts in food processing and the often lengthy and time consuming process of having to perform individual nut food challenges.

However, clinical practice is now evolving with prevention strategies for food allergy around delaying the introduction of common allergens failing to show a benefit. Further a recent systematic review and meta-analysis summarising results of RCTs exploring timing of introduction of allergenic solids and food allergy outcomes supports the protective effect of earlier introduction of common allergenic foods[9]. Additionally, tree nuts have become an increasingly common part of our diet and there is increasing acknowledgement of the cardiovascular health benefits of consuming nuts.[10-12] The culmination of this is clinical management of peanut and tree nut allergy is evolving and we can no longer base our assumptions of tree nut allergy on what we know of peanut allergy.

This thesis aims to explore the prevalence, elements of the natural history and development of tree nut allergy and the frequency of adverse food reactions related to tree nuts in the population-based HealthNuts and SchoolNuts studies and the Royal Children's Hospital allergy clinic populations. This thesis has the following objectives.

1.2 Objectives

1.2.1 Tree nut allergy prevalence

1. What is the worldwide prevalence of tree nut allergy?
2. Does tree nut allergy prevalence vary by region?
3. What are the most common individual tree nut allergies around the world?

In the HealthNuts study:

4. What is the prevalence of tree nut allergy in Australian children aged 1 and 6 years of age?
    a) What is the prevalence of parent-reported tree nut allergy at age 1 year?
    b) What is the prevalence of challenge-confirmed tree nut allergy at age 6 years?
    c) What are the most common individual tree nut allergies in children at age 6 years?

1.2.2 Tree nut allergy adverse events

In the SchoolNuts study:

1. Do food allergic sufferers have more reactions or more severe reactions when peanut or tree nut allergic versus other food allergies?
    a) What is the prevalence of self-reported adverse reactions for peanut, tree nut and other foods in 10 to 14 year old allergic children?
    b) What is the prevalence of self-reported anaphylaxis for peanut, tree nut and other foods in 10 to 14 year old allergic children?
    c) What factors are associated with adverse food allergy reactions and anaphylaxis in 10 to 14 year old allergic children?

1.2.3 Development of tree nut allergy

In the HealthNuts study:

1. What proportion of those with challenge-confirmed food allergy at age one year are tree nut sensitised?
2. What is the relationship between tree nut sensitisation at age one year and tree nut allergy at age 6 years?
3. What is the relationship between food allergy type at age one year and development of tree nut allergy at age 6 years?
4. What is the frequency of co-allergy to peanut and other nuts at age 6 years?

1.2.4 Cashew nut oral food challenge outcomes

1. What are the SPT wheal sizes that correlate with a 95% positive predicative value (PPV) of a positive oral food challenge for cashew
2. Do these thresholds differ when stratified by allergy clinic or general population cohorts?
3. Do cashew SPT thresholds differ when stratified by known risk factors including co-existing peanut allergy, coexisting other food allergies, coexisting atopy, previous reaction history, age and gender.

2.LITERATURE REVIEW

This chapter firstly provides a broad overview of food allergy diagnosis, treatment and risk factors and then secondly outlines the background literature specific to the research questions addressed in this thesis regarding tree nut allergy prevalence, natural history and adverse events.

2.1 Food Allergy Overview

Food allergy is common, affecting up to 10% of children and 2-3% of adults, and appears to be increasing in prevalence.[2-4,13]

Food allergy is characterized by an adverse reaction to food proteins, mediated by immunological mechanisms.[14] Symptoms occur quickly and can include multiple body systems. Food allergies can occur to any food, however in children 90% of all reactions are due to one of eight foods: cow's milk, egg, wheat, soy, seafood, fish, peanuts and tree nuts.[5]

Food allergies are generally divided into two types – immediate (Immuno-globulin E or IgE-mediated) and delayed (non IgE-mediated). IgE-mediated reactions are more common and can be life threatening (Figure 2.1).

Cow's milk and egg are reported as the most common IgE-mediated food allergies in infants and young children, however the prognosis is good with resolution expected for the majority of children by school age.[15] Peanut and tree nut allergies on the other hand tend to be lifelong and are the cause of most food related anaphylaxis and deaths.[16]

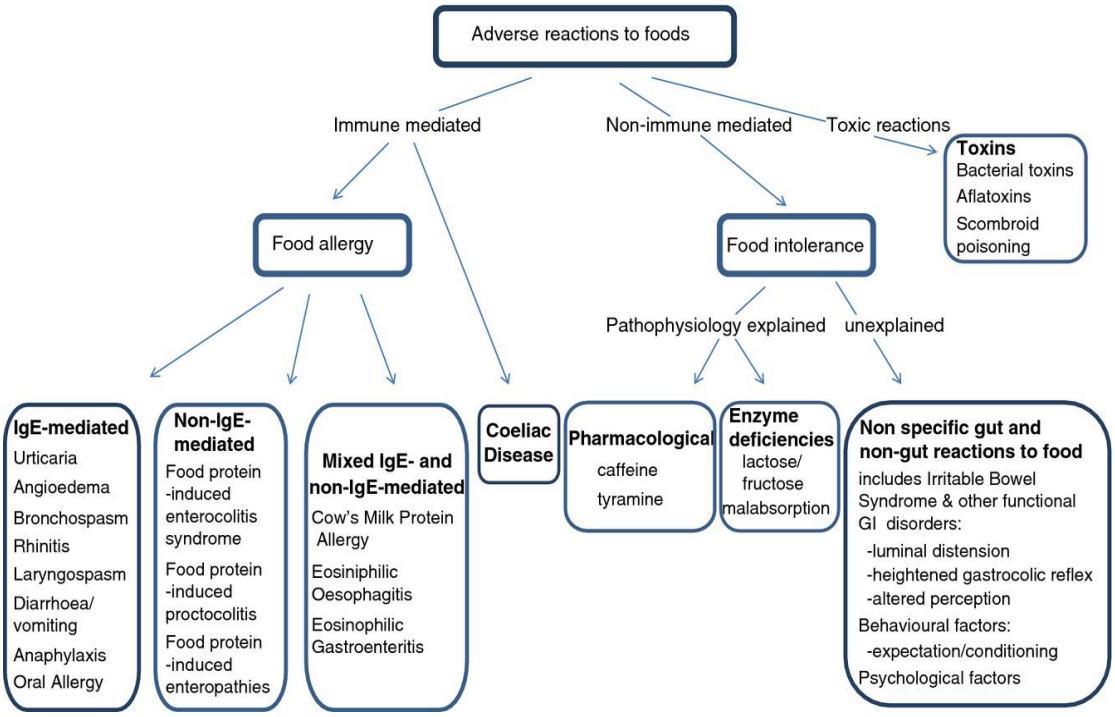


Figure 2.1. Classification of adverse reactions to foods

Sourced with permission from Turnbull JL, et al. Review article: the diagnosis and management of food allergy and food intolerances. *Alimentary Pharmacology & Therapeutics.* 2015; 41(1):3-25.[17]

2.1.1 Mechanism of action/symptoms

The gastrointestinal mucosa is the principal site for the immune systems' interaction with any ingested substances and consists of a single layer of columnar epithelium. The enteric immune system has the important task of policing the mucosal boundary distinguishing necessary and harmless food proteins and commensal enteric microbiota from potentially harmful pathogens.[14] This process is known as oral tolerance and to date the precise mechanisms of oral tolerance are not fully understood.[18] IgE-mediated allergic reactions occur when there is a breakdown of oral tolerance mechanisms and the mucosal immune system responds inappropriately to ingested food proteins. Absorption of food proteins through the intestinal epithelium and access to the mucosa and blood stream where immune effector cells reside is thought to be one step in the breakdown of oral tolerance mechanisms and is enhanced in those with food allergy.[19]

The allergic response is separated into two phases, the sensitisation phase and the effector phase. Sensitisation is the production of IgE-antibodies to usually harmless food antigens.[20] The food protein is digested by the gastrointestinal tract where some proteins may enter the blood stream. Antigen-presenting cells present the circulating proteins to T cells stimulating the production of specific cytokines. T cells in turn interact with B cells and induce the production of food-specific IgE antibodies. These antibodies then circulate in the bloodstream and bind to receptors on mast cells in the gastrointestinal tract, skin and respiratory tract. The presence of food-specific IgE antibodies is called sensitisation. Sensitisation is a harmless process and a person can be sensitised with no clinical signs of food allergy on ingestion of the food proteins to which they are sensitised. The effector phase occurs at subsequent exposures where food antigens bind to IgE receptors on the surface of mast cells. This causes degranulation and the release of inflammatory mediators and metabolites from the mast cells. These mediators and metabolites can act locally in the gastrointestinal tract or be distributed systemically resulting in a broad range of symptoms (Figure 2.2).[14] It remains unknown how and where food sensitisation occurs, however several RCTs exploring introduction of food allergens for food allergy prevention have shown sensitisation to food allergens prior to any known ingestion in the infants diet[21-23] suggesting exposure through breast milk or the placenta. An alternate suggestion is that dermal contact of food antigens can be a route of sensitisation.[24]

IgE-mediated reactions usually occur within minutes of ingestion (although they can occur up to 1-2 hours after exposure) and result in a range of symptoms that can involve the skin, gastrointestinal, respiratory or cardiovascular systems.[25] Gastrointestinal symptoms can include itching or tingling of the tongue and lips, tightness in the throat, nausea, vomiting, diarrhoea and abdominal cramps. Cutaneous symptoms include urticarial lesions (hives), pruritus, angioedema and eczema flare. The most severe allergic reaction is anaphylaxis, which can be fatal. Anaphylaxis involves multiple body systems and symptoms may include wheeze, bronchospasm, hypotension and shock.[17] The combination of symptoms used to define anaphylaxis vary slightly among key allergy bodies around the world [26-29] (Table 1).

Table 1. Anaphylaxis Definitions

Agency	Anaphylaxis Definition
National Institute of Allergy and Infectious Diseases (NIAID) and the Food Allergy and Anaphylaxis Network (FAAN) (NIAID/FAAN) (Sampson et al, 2006)[26]	Anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled: 1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g. generalized hives, pruritus or flushing, swollen lips-tongue-uvula) AND AT LEAST ONE OF THE FOLLOWING a. Respiratory compromise (e.g., dyspnoea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia) b. Reduced BP or associated symptoms of end-organ dysfunction (e.g. hypotonia [collapse], syncope, incontinence) OR 2. Two or more of the following that occur rapidly after exposure to a likely allergen for that patient (minutes to several hours): a. Involvement of the skin-mucosal tissue (e.g. generalized hives, itch-flush, swollen lips-tongue-uvula) b. Respiratory compromise (e.g. dyspnoea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia) c. Reduced BP or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence) d. Persistent gastrointestinal symptoms (e.g. crampy abdominal pain, vomiting) OR 3. Reduced BP after exposure to known allergen for that patient (minutes to several hours): a. Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP* b. Adults: systolic BP of less than 90 mmHg or greater than 30% decrease from that person's baseline *PEF, Peak expiratory flow; BP, blood pressure;*Low systolic blood pressure for children is defined as less than 70 mm Hg from 1 month to 1 year, less than (70 mm Hg+ [2×age]) from 1 to 10 years, and less than 90 mm Hg from 11 to 17 years.*
World Allergy Organization (WAO) (Simons et al, 2011)[27]	Anaphylaxis is highly likely when any one of the following 3 criteria is fulfilled 1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized urticarial, itching or flushing, swollen lips-tongue-uvula) AND AT LEAST ONE OF THE FOLLOWING: a. Respiratory compromise (e.g., dyspnoea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia) b. Reduced blood pressure or associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope, incontinence) OR 2. Two or more of the following that occur rapidly after exposure to a likely *allergen*[a] for that patient (minutes to several hours) a. Involvement of the skin-mucosal tissue (e.g., generalized urticaria, itch-flush, swollen lips-tongue-uvula) b. Respiratory compromise (e.g., dyspnoea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia) c. Reduced blood pressure or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence) d. Persistent gastrointestinal symptoms (e.g., crampy abdominal pain, vomiting) OR 3. Reduced blood pressure after exposure to known *allergen*[b] for that patient (minutes to several hours) a. Infants and children: low systolic blood pressure (age-specific) or greater than 30% decrease in systolic blood pressure[c] b. Adults: systolic blood pressure of less than 90 mm Hg or greater than 30% decrease from that person's baseline *PEF: peak expiratory flow.* *a. Or other trigger, for example, immunologic but IgE-independent, or non-immunologic (direct) mast cell activation.* *b. For example, after an insect sting, reduced blood pressure might be the only manifestation of anaphylaxis; or, in a similar example, during allergen immunotherapy, after injection of a known allergen for that patient, generalized urticaria (only one body organ system affected) might be the only initial manifestation of anaphylaxis.* *c. Low systolic blood pressure for children is defined as less than 70 mm Hg from 1 month to 1 year, less than (70 mm Hg +[2×age]) from 1 to 10 years, and less than 90 mm Hg from 11 to 17 years. Normal heart rate ranges from 80–140 beats/min at age 1–2 years; from 80–120 beats/min at age 3 years; and from 70–115 beats/min after age 3 years. Infants are more likely to have respiratory compromise than hypotension or shock, and in this age group, shock is more likely to be manifest initially by tachycardia than by hypotension.*

Agency	Anaphylaxis Definition
European Academy of Allergy and Clinical Immunology (EAACI) (Muraro et al, 2014)[30]	Anaphylaxis is highly likely when any one of the following 3 criteria is fulfilled 1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, pruritus or flushing, swollen lips-tongue-uvula) AND AT LEAST ONE OF THE FOLLOWING: a. Respiratory compromise (e.g., dyspnoea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia) b. Reduced BP or associated symptoms of end-organ dysfunction (e.g. hypotonia [collapse], syncope, incontinence) 2. Two or more of the following that occur rapidly after exposure to a likely *allergen* for that patient (minutes to several hours) a. Involvement of the skin-mucosal tissue (e.g., generalized hives, itch-flush, swollen lips-tongue-uvula) b. Respiratory compromise (e.g., dyspnoea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia) c. Reduced blood pressure or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence) d. Persistent gastrointestinal symptoms (e.g., crampy abdominal pain, vomiting) 3. Reduced BP after exposure to known *allergen* for that patient (minutes to several hours) a. Infants and children: low systolic BP (age-specific) or>30% decrease in systolic BP* b. Adults: systolic BP of < 90 mm Hg or > 30% decrease from that person's baseline *PEF, peak expiratory flow; BP, blood pressure* **Low systolic blood pressure for children is defined as <70 mm Hg from 1 month to 1 year, less than (70 mm Hg + [2×age]) from 1 to 10 years, and < 90 mm Hg from 11 to 17 years.*
Australasian Society of Clinical Immunology and Allergy (ASCIA) (ASCIA, 2016)[29]	Any acute onset of hypotension or bronchospasm or upper airway obstruction where anaphylaxis is considered possible, even if typical skin features are absent. OR Any acute onset illness with typical skin features (urticarial rash or erythema/flushing, and/or angioedema), with respiratory compromise due to bronchospasm or significant upper tongue/throat swelling and/or cardiovascular symptoms. In those with severe allergic reactions to insect stings, the presence of severe abdominal pain and vomiting may also indicate anaphylaxis, since their presence correlates with the presentation of hypotension in this group. Symptoms/signs of respiratory/cardiovascular involvement include: 1. Respiratory: a. Difficult/noisy breathing b. Swelling of tongue c. Swelling/tightness in throat d. Difficulty talking and/or hoarse voice e. Wheeze or persistent cough 2. Cardiovascular: a. Loss of consciousness/collapse b. Persistent dizziness c. Pale and floppy (in young children) d. Hypotension e. Loss of vision

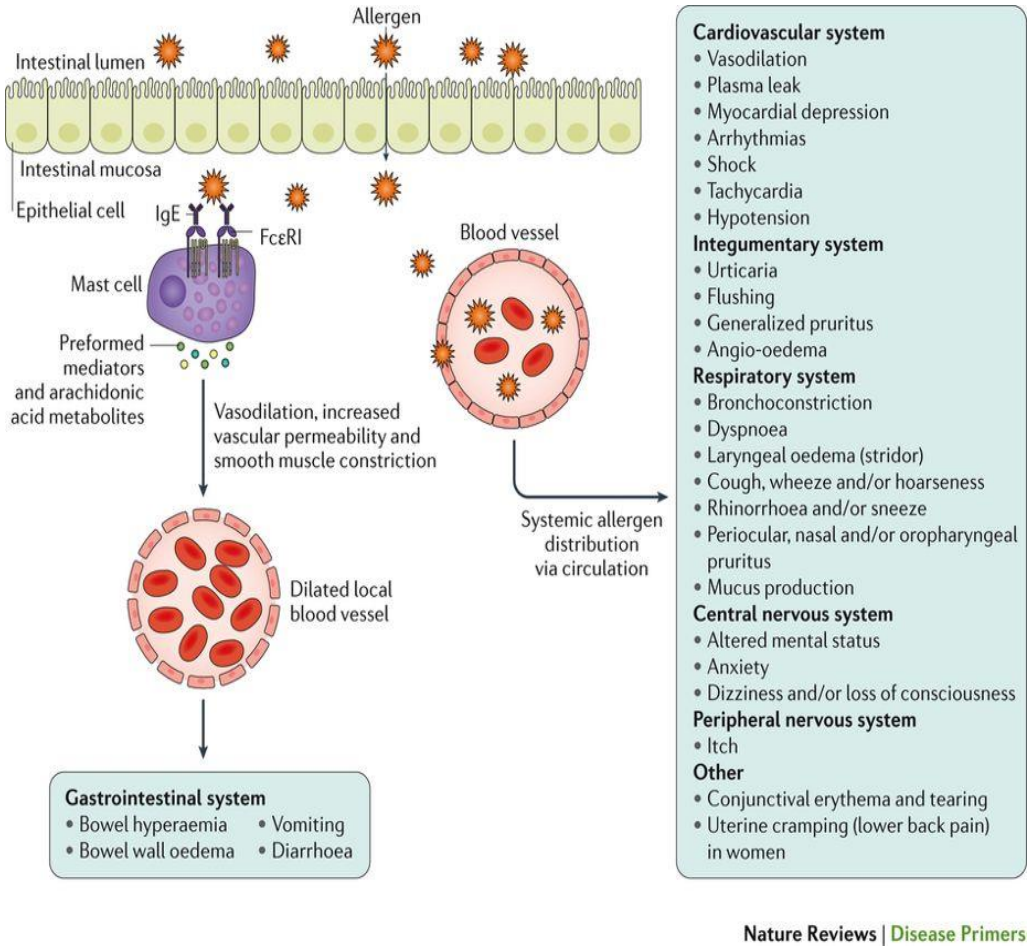


Figure 2.2. Mechanism of IgE-mediated food allergy reactions and symptoms

Sourced with permission from Renz H, Allen KJ, Sicherer SH, et al. Food allergy. *Nat Rev Dis Primers.* 2018;4:17098.[14]

2.1.2 Food Allergy Diagnosis

Food allergy diagnosis is complex with no single diagnostic test providing a definitive diagnosis. Clinically, diagnosis is based on a stepped approach with the starting point a detailed medical history and physical examination which guides diagnostic testing for specific IgE levels. When necessary OFCs are used for confirmation. Double-blind placebo-controlled food challenges are recognised as the gold standard for clinical diagnosis.[31]however due to the time and labour-intensive nature of these tests, open food challenges are typically performed in the clinical setting. Figure 2.4 outlines the diagnosis of food allergy.

Medical History

Key aspects of the medical history that point to a reaction being IgE-mediated include the time interval between exposure and onset of symptoms, the type of symptoms experienced, duration of symptoms and the type of food[32] (refer to page 7 for a description of IgE mediated food allergy symptoms). Mild reactions, such as hives or skin rashes, can occur from touching foods. However, a contact reaction that is localised is not diagnostic of a food allergy and an ingestion reaction is required to confirm allergy.[25]

Specific IgE testing

In the presence of a history suggestive of a reaction, IgE testing can confirm that the adverse reaction is likely IgE-mediated through the detection of allergen-specific IgE either in the skin via skin prick testing (SPT) or blood (serum specific IgE, sIgE). IgE testing has limitations, firstly a positive result merely indicates the presence of IgE antibodies. Without a history of a reaction, IgE testing cannot distinguish between clinically allergic versus sensitised tolerant.[31]Negative results however, are valuable for excluding food allergy with greater than 95% certainty.[33] Secondly, IgE levels can only be used to predict the likelihood of a reaction not the severity of a reaction. SPTS are generally performed in preference to sIgE in most tertiary paediatric allergy clinics in Australia as the results are immediately available.

SPT involves introducing small amounts of allergen into the epidermis which interact with specific IgE bound to cutaneous mast cells.[33] SPTs can be performed either with commercial extracts or with fresh foods. A positive result occurs within 15-20minutes when histamine and other mediators are released leaving a visible “wheal” reaction which is measured and results expressed as the mean diameter in millimetres (mm).A positive SPT is generally considered a wheal with a mean diameter of 3mm or greater than the negative control.[30]

Oral Food Challenge

Oral food challenges (OFC) remain the most definitive diagnostic method for food allergy. They are performed by feeding the suspected allergen, in gradual increasing amounts, under medical supervision. OFC are usually performed to determine if a known food allergy has resolved or in the context of positive allergy testing and no known history of reaction or ingestion of the suspected allergen. An OFC can be performed as either an open challenge (both the clinician performing the challenge and the patient are aware of the challenge food), a single-blind placebo-controlled food challenge (the clinician is aware of when allergen or

placebo is being administered but not the patient) or the gold standard double-blind placebo-controlled food challenge (DBPCFC) (both clinician and patient are unaware of when the active or placebo challenge is performed). A DBPCFC removes measurement and reporting bias for the observer and the psychological effect from the patient. To date DBPCFC are only preformed in some allergy centres or limited to research purposes due to labour, time and cost constraints. There is huge variability among allergy centres and research studies regarding OFC protocols used and the symptoms that correlate with a positive food challenge. In 2008, a group of food allergy experts from Europe and the United States proposed a standard procedure for conducting DBPCFC with the aim of standardising the procedure for research purposes but this is also a useful framework applicable to open OFCs used in the clinical setting (Figure 2.3).[34]

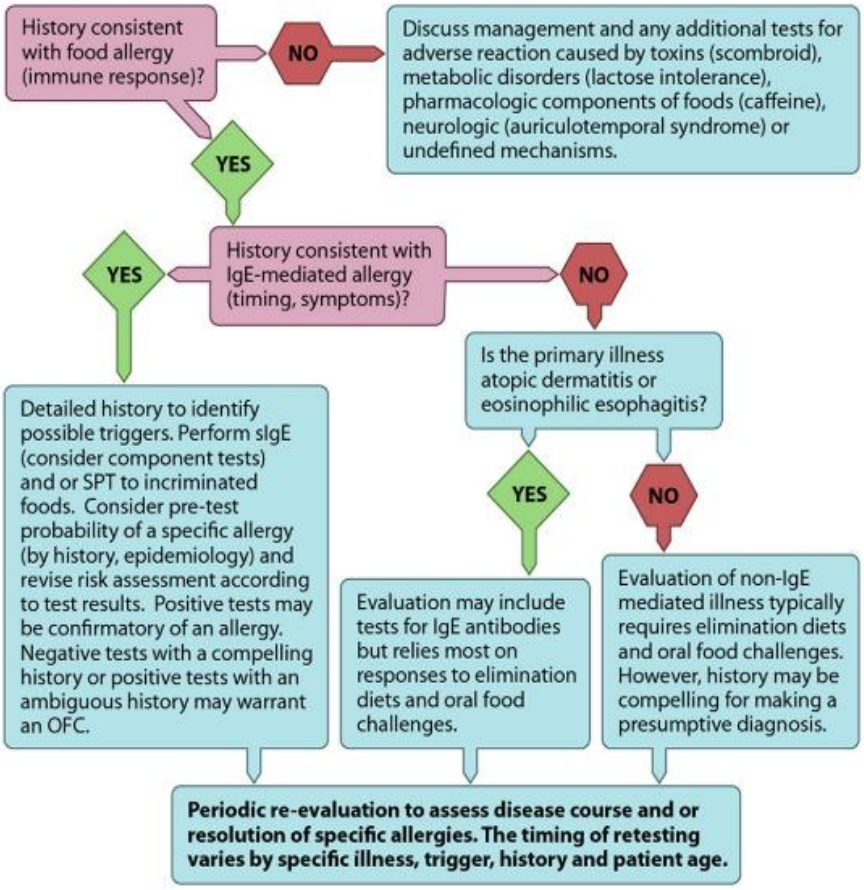


Figure 2.3. Algorithm for the diagnosis of food allergy

Sourced with permission from Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *The Journal of Allergy and Clinical Immunology.* 2018;141(1):41-58.[35]

I. SKIN

A. Erythematous Rash- % area involved_____

B. Pruritus
0 = Absent
1 = Mild, occasional scratching
2 = Moderate -scratching continuously for > 2 minutes at a time
3 = Severe – hard continuous scratching – excoriations

C. Urticaria/Angioedema
0 = Absent
1 = Mild – < 3 hives, or mild lip edema
2 = Moderate - < 10 hives but >3, or significant lip or face edema
3 = Severe – generalized involvement

D. Rash
0 = Absent
1 = Mild – few areas of faint erythema
2 = Moderate – areas of erythema
3 = Severe – generalized marked erythema (>50%)

II. UPPER RESPIRATORY

A. Sneezing/Itching
0 = Absent
1 = Mild – rare bursts, occasional sniffing
2 = Moderate – bursts < 10, intermittent rubbing of nose, and/or eyes or frequent sniffing
3 = Severe – continuous rubbing of nose and/or eyes, periocular swelling and/or long bursts of sneezing, persistent rhinorrhea

III. LOWER RESPIRATORY

A. Wheezing
0= Absent
1 = Mild – expiratory wheezing to auscultation
2 = Moderate – inspiratory and expiratory wheezing
3 = Severe – use of accessory muscles, audible wheezing

B. Laryngeal
0= Absent
1 = Mild – >3 discrete episodes of throat clearing or cough, or persistent throat tightness/pain
2 = Moderate – hoarseness, frequent dry cough
3 = Severe – stridor

IV. GASTROINTESTINAL

A. Subjective Complaints
0 = Absent
1 = Mild–complaints of nausea or abdominal pain, itchy mouth/throat
2 = Moderate – frequent c/o nausea or pain with normal activity
3 = Severe - notably distressed due to GI symptoms with decreased activity

B. Objective Complaints
0 = Absent
1 = Mild – 1 episode of emesis or diarrhea
2 = Moderate – 2-3 episodes of emesis or diarrhea or 1 of each
3 = Severe – >3 episodes of emesis or diarrhea or 2 of each

V. CARDIOVASCULAR/NEUROLOGIC

0 = normal heart rate or BP for age/baseline
1 = mild-subjective response (weak, dizzy), or tachycardia
2 = moderate-drop in blood pressure and/or >20% from baseline, or significant change in mental status.
3 = severe-cardiovascular collapse, signs of impaired circulation (unconscious)

TABLE LEGEND:

GREEN:
- Not usually an indication to alter dosing.
- Not generally sufficient to consider a challenge positive.

Orange (scores increasing to orange):
- Caution, dosing could proceed, be delayed, have a dose repeated rather than escalated.
- If clinically indicated, dosing is stopped.
- Symptoms that recur on 3 doses, or persist (e.g., 40 minutes) are more likely indicative of a reaction than when such symptoms are transient and not reproducible.
- 3 or more scoring areas in orange more likely represent a true response.

RED:
- Objective symptoms likely to indicate a true reaction
- Usually an indication to stop dosing.

Figure 2.4. Standardised stopping criteria for oral food challenges

Sourced with permission from Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, 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. The Journal of allergy and clinical immunology. 2012;130(6):1260-1274[34]

2.1.3 Improving Food Allergy Diagnosis

There remains significant limitations to food allergy diagnosis. Current clinical diagnostic tools of sIgE testing cannot reliably determine those patients that are clinically allergic from those that are sensitised tolerant. Diagnosis is reliant on time intensive, costly and potentially risky OFC. The application of 95% PPV cut-off values to current IgE testing and the use of *invitro* techniques such as component resolved diagnostics and basophil activation testing are all potential options for improvements in food allergy diagnosis and each will be discussed in further detail in the following sections.

The diagnostic value of SPT for food allergy diagnosis

The diagnostic value of a test is determined by comparing the test results to that of the gold standard. With regards to food allergy and the test studied in this thesis, the standard test is the SPT and the gold standard is the OFC. The diagnostic value of a test is typically determined based on the statistics described below and can be summarised using a two-by-two table (Table 2).

Table 2. Determining the diagnostic value of a test

Gold Standard (Oral food Challenge)			
	With Disease (Food allergic)	Without Disease (Tolerant/Not allergic)	
Test positive (SPT)	True positive a	False positive b	PPV=a/(a+b)
Test negative (SPT)	False negative c	True negative D	NPV = d/(c+d)
	Sensitivity =a/(a+c)	Specificity =d/(b+d)	
Positive likelihood ratio= sensitivity/(1-specificify) Negative likelihood ratio= (1-senstivity)/specificity			

Sensitivity: the ability of a diagnostic test (SPT) to correctly classify an individual as having a disease (allergic).

Specificity: the ability of a diagnostic test (SPT) to correctly classify an individual as tolerant or not allergic.

Likelihood Ratios: combines sensitivity and specificity and provide a summary of how many times more/or less likely patients with food allergy are to have a particular SPT than patients without food allergy.

Positive predictive value (PPV): the percentage of patients with a positive SPT who are actually allergic. The higher the percentage the closer the test (SPT) is to the gold standard test (OFC).

Negative predictive value (NPV): the percentage of patients with a negative SPT who are not allergic. The higher the percentage the closer the test (SPT) is to the gold standard test (OFC).

To estimate sensitivity and specificity, each individual needs to be classified definitively via a gold standard test (OFC) as a true positive (allergic) or true negative (tolerant/not allergic) and, in addition, be classified according to the test being assessed (SPT). SPT has high sensitivity, but low specificity to clinical food allergy. This means that while SPT is accurate in identifying allergen-specific IgE, which will be present in all individuals with IgE-mediated food allergy, detectable SPT does not necessarily equate to clinical food allergy, as some children are sensitised yet able to tolerate the food.[31] Negative results however, are valuable for excluding food allergy with greater than 95% certainty.[33]

Sensitivity and specificity describe the quality of the test, they do not describe an individual patient's risk of the outcome and therefore have limited clinical usefulness. To interpret the results of an SPT a clinician will want to know the probability that a patient is truly allergic if the test is positive (positive predictive value) and similarly the probability that the patient is truly tolerant if the test is negative (negative predictive value).

As SPT results are quantitative and the magnitude of the test is correlated to the risk of clinical reaction,[31] diagnostic cut-offs (SPT wheal size) that have 95% positive predictive value to clinical food allergy can be determined and have been reported for some common food allergens.[36-47] These values vary for different allergens and across different studies however, 95% PPVs have been adopted as a tool for clinicians to more accurately determine when an OFC should be performed. In general terms a SPT wheal of 8mm is commonly used as the 95%PPV for clinical food allergy. There are however several methodological considerations that will impact the pre-test probability of food allergy and therefore the reliability of PPVs such as age, food allergy definition, SPT method and the population SPTs are performed on hence the variability in figures reported.[48]

A common limitation of allergy studies is they recruit participants from high-risk settings such as children attending tertiary allergy clinics, as it is an easy and feasible means to recruit a large sample of cases. However, children recruited from one clinic setting may not be generalisable to another clinic and allergy clinic cohorts will have higher allergy prevalence than those of community based settings. Secondly, food allergy research can include variable definitions of food allergy. Relatively few studies are based on the gold standard OFC due to cost or impracticalities. Studies instead may rely on suggestive clinical history, SPT or sIgE levels or self-reporting. These measures are known to overestimate the prevalence of food

allergy, and in turn may bias the association between predictors and food allergy.[2,4] In particular, SPT or sIgE thresholds used as a proxy for food allergy diagnosis in studies assessing the predictive value of these tests, significantly limits the value of these studies and overestimates the PPV.[49] Although SPT wheal thresholds with high predictably have been reported for several common food allergens these values should not be applied to populations where there are differences in population characteristics, allergy prevalence, allergy definitions and SPT methods from the population in the published study.[50]

To overcome the methodological limitations of predictive values, likelihood ratios (LR) can be used as they are independent of disease prevalence. Likelihood ratios provide a summary of how many times more (or less) likely patients with a disease are to have a particular result than patients without the disease.[49] However, the calculation and application of likelihood ratios is complex and this has limited the use in clinical practice. It requires clinicians determining a pre-test (SPT) probability of their patient having food allergy based on clinical history and risk factors. The SPT result is then used to modify the pre-test probability of having food allergy. A positive SPT may increase the pre-test probability and a negative test may reduce the pre-test probability. The patient's probability of allergy after SPT is the post-test probability. To utilise likelihood ratios in clinical practice a graphical tool known as a Fagan's nomogram is needed which allows the pre-test probability in conjunction with the LR, to determine the patient's' post-test probability i.e. their probability of being clinically allergic.[49,51]

It has been proposed that likelihood ratios should be the test applied to determine the probability of allergic disease,[49] however PPVs remain more commonly used in clinical practice. It has been previously reported that PPVs for sIgE derived from clinic populations cannot be meaningfully applied to general populations. To date this has not been explored for SPT.[52] A compromise may be the estimation of PPVs in population-based settings, utilising OFC outcomes, therefore addressing the impact of selection bias and allergy definition on prevalence estimates and PPVs reported in previous research.

Component Resolved Diagnostics

Routine SPT and sIgE rely on crude food extracts and do not differentiate which particular proteins within a food are interacting with a patient's IgE antibody and driving the allergic response. Advances in protein purification and molecular biology techniques have led to an

improved understanding of which particular proteins are more strongly associated with clinical reactivity. Component resolved diagnostic tests (CRD) determine the individual allergen to which a patient's IgE is directed. Whilst 100% efficacy has not been demonstrated for CRD and the gold standard for allergy diagnosis remains an OFC, a number of studies have demonstrated that CRD may improve the specificity of allergy testing to a variety of food allergens with peanut allergy the most extensively studied to date.[53] There are at least ten known sub-components of the peanut allergen with Ara h 2 the predominant allergen.[54,55] For patients with SPT and/or sIgE levels that indicate a likelihood of allergy resolution, Ara h 2 levels can be measured to further inform the decision whether to proceed to an OFC. Dang et al reported Ara h 2 sIgE levels were more accurate in determining peanut allergy compared to whole peanut sIgE levels. An Ara h 2 s IgE level of 0.46 kU_A/l provided 95 % specificity and 73 % sensitivity, whereas a peanut sIgE level of 6.2 kU_A/l provided 95 % specificity, but only 44 % sensitivity. When using a peanut sIgE level of 15 kU_A/l, providing a 95% PPV and 98% specificity, the sensitivity of the peanut sIgE drops to 26 %. An Ara h 2 level of 1.19 kU_A/l was also found to provide 98% specificity, but offered a much better sensitivity of 60%. Given the improved accuracy found in the Ara h 2 sIgE diagnostic testing, they concluded that this test should be considered the preferred diagnostic tool for determining peanut allergy.[56]

Whilst CRD is a very significant development in food allergy research and management and has the potential to help determine those individuals who have a higher likelihood of severe reactions, multiple food allergies or more persistent food allergies it is not yet an alternative to existing methods of allergy testing, as it not as sensitive, not widely available, and investegations of component testing for a number of major food allergens (including tree nuts) are lacking. The application of CRD for tree nut allergy is discussed further in section 2.2.7.

Basophil Activation Testing (BAT)

A second invitro technique has also been explored for food allergy diagnosis known as the basophil activation test (BAT). Basophils are the mast cells involved in acute allergic reactions. The BAT is a flow cytometry-based assay where the expression of activation markers is measured on the surface of basophils following simulation with an allergen.[57] The performance of BAT as a measure of food allergy has been tested most extensively for peanut allergy to date[58-62] and has shown high specificity (96%) and 95% positive predictive value[59]. The advantages of BAT are that it can be used for patient not suitable for SPT due to with extensive eczema or recent antihistamine medication and it has a reported high safety profile.[63]The

downsides are the need for fresh blood and the cost of resources and technical expertise to perform.[57] The diagnostic accuracy and superiority over SPT and sIgE needs to be assessed with other allergens and in a wider range of clinical settings but again is another significant development in food allergy research and management.

For now the diagnosis of food allergy is limited to specific IgE and SPT based on crude food extracts with OFC to confirm diagnosis for those that are sensitised. Further research is required to improve our understanding of the application of additional *invitro* tests such as CRD and BAT for food allergy diagnosis.

2.1.4 Food Allergy Treatment

To date, treatment options for food allergy remain limited and primarily involve allergen avoidance and administration of medical treatment in the event of an accidental exposure, such as antihistamines and adrenaline.[64]

On receiving a diagnosis of food allergy, parents or guardians should receive comprehensive advice on allergen avoidance, interpretation of food allergen labelling and identification of potential sources of cross-contamination. Advice regarding adequate replacement of excluded foods to ensure nutritional adequacy of the diet is also important as poor growth and nutritional deficiencies in children with food allergies, particularly multiple food allergies has been well documented.[65-74] Therefore, parents and guardians should receive appropriate education and for those with multiple food allergies in particular, referral for management by a dietician.[73]

Clinical trials are underway exploring new therapeutic treatments for food allergy with most of the work focusing on systemic, epicutaneous, subcutaneous, sublingual and oral immunotherapy (OIT). Immunotherapy is a process by which desensitisation to the allergen and development of oral tolerance is sought through continuous low-dose exposure to the allergen. All forms of immunotherapy have been shown to improve a patient's reaction threshold and/or achieve desensitisation in the context of ongoing ingestion of the allergen however, the challenge is achieving true oral tolerance (sustained unresponsiveness). That is, an ability to not react to the allergen even after periods of not being exposed.[75,76]The

downsides of all forms of immunotherapy for food allergy to date are the intensive treatment regimens and particularly for OIT the high frequency of significant allergic reactions and gastrointestinal symptoms reported.[64] The balance of allergic side effects and the need for ongoing ingestion of the allergen versus the protection from severe reactions will be an individual decision for each patient and his or her family. Further work is required to evaluate long term effectiveness and safety before immunotherapy for food allergy is available for clinical application.

2.1.5 Risk factors for development of food allergy

Over the past decade, the risk factors for developing food allergy have become better understood. Factors both intrinsic to the individual and related to environmental exposures appear to influence the development of allergy and current evidence suggests that the risk is multifactorial. Evidence currently centres around three main hypotheses: 1. the vitamin D hypothesis; 2. the hygiene hypothesis; and 3.the dual-allergen exposure hypothesis.

1. The vitamin D hypothesis

Ecological and epidemiological evidence suggest a potential link between low vitamin D and allergy risk.[77] A strong latitude and food allergy association has been observed with regions further from the equator, a proxy for low vitamin D levels, reporting higher rates of food allergy, adrenalin-auto injector prescriptions and hospital admissions for food-related anaphylaxis.[78-85]Season of birth (eg winter), another proxy for vitamin D exposure has also been associated with increased risk of eczema, food allergy and adrenalin auto-injector prescriptions.[86-89]

Indirect evidence studies reporting on vitamin D levels and the risk of allergies have produced conflicting results. The Australian HealthNuts study, provided the first evidence that vitamin D deficiency was associated with an increased risk of food allergy, reporting that vitamin D insufficiency (<50nmol) in the first year of life increased the risk of challenge confirmed peanut allergy at age 1 year by 11-fold (aOR 11.51; 95%CI, 2.01-65.79) and egg allergy nearly 4-fold (aOR 3.79; 95%CI 1.19-12.08) among those infants with Australian-born parents.[90]

In contrast, a smaller case-control study also in Australia did not find an association between Vitamin D insufficiency and food allergy.[91]While a cross-sectional study in New Zealand

reported a 2-fold increased risk of parent-reported, doctor-diagnosed food allergy in pre-school aged children with vitamin D levels >75nmol/l, compared to those with levels between 50-74nmol.[92] This observation has led to the suggestion of a U-shaped association, that is, an increased risk in individuals with both low and high levels of vitamin D.[93]

The underlying mechanisms of vitamin D and food allergy development are not yet understood, largely due to limited studies collecting well defined clinical measures of food allergy, along with serum vitamin D measures and detailed immunological responses. There is however some evidence to suggest that vitamin D is important in several immune functions, particularly in the development of effector T cell subsets.[94]Work from the HealthNuts study has revealed that immune responses associated with the development of oral tolerance to foods in childhood positively correlated with increased serum vitamin D supplementation.[95]

There are RCTs addressing supplementation in pregnancy and lactation on allergic outcomes[96-99] and one study underway in infants, the VITALITY study.[100]However, to date there is a lack of direct evidence to guide recommendations regarding vitamin D supplementation for food allergy prevention. Interestingly there has been recent work published that suggests that direct UV exposure appears more beneficial than vitamin D supplementation in early infancy but this also requires further research.[101]

2. The hygiene hypothesis

The hygiene hypothesis proposes that a lack of exposure to microbes and infections in childhood increases susceptibility to allergic disease via modulation of the developing immune system.[102,103] Observational studies have shown that factors associated with increased microbial exposure, such as exposure to pets[104] or farming environments[105-108], the presence of older siblings or communal childcare attendance[103,104,107] and mode of delivery[109] may have protective effects on the development of food allergy.

These assumptions are further supported by studies showing significant differences in the gut microbiota profiles between allergic and non-allergic infants and children.[110-112] However data directly characterising the microbiota of patients with food allergy are still preliminary. At this time there is no direct evidence to support the use of specific nutritional or dietary factors to manipulate the gut microbiome and immune system to prevent or treat food allergy at this time.[111]A summary of microbial factors and risk of food allergy are summarised in Figure 2.5.

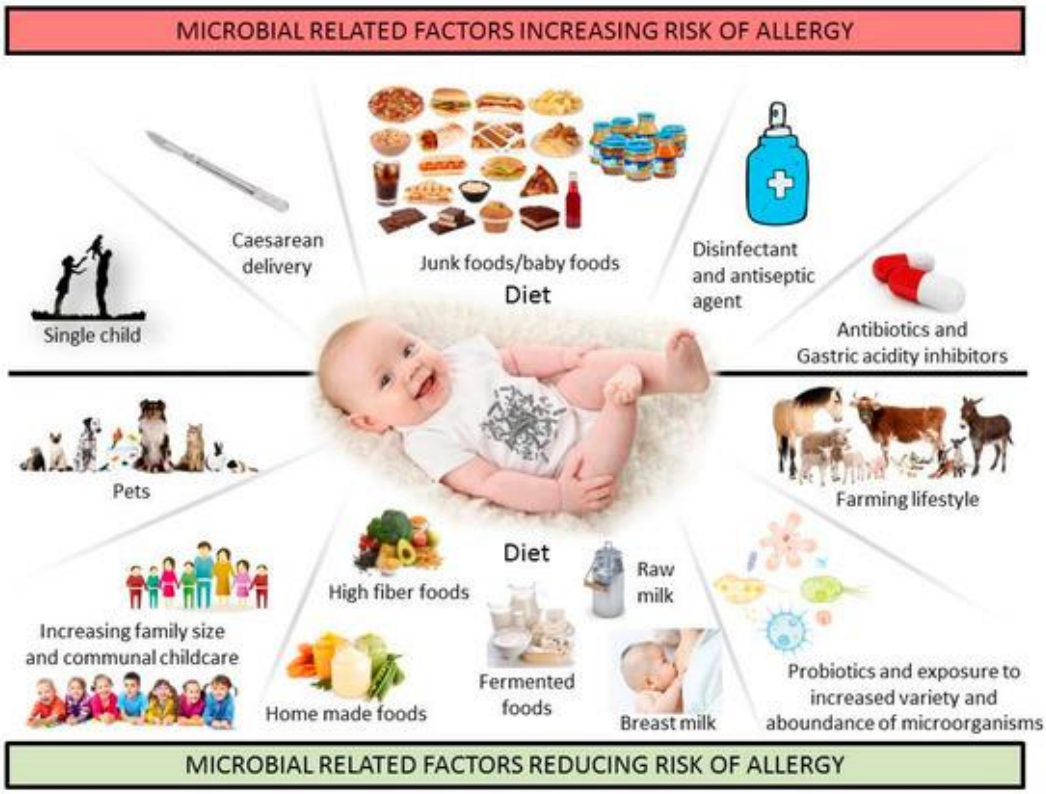


Figure 2.5. Environmental and lifestyle factors related to microbial exposure and their effect on the risk of developing food allergy

Sourced with permission from Aitoro R, Paparo L, Amoroso A, et al. Gut Microbiota as a Target for Preventive and Therapeutic Intervention against Food Allergy. *Nutrients.* 2017; 9(7).[113]

3. The dual-allergen exposure hypothesis

The dual-allergen exposure hypothesis proposes that sensitisation to food allergens may occur through low-dose cutaneous exposure in infants with altered skin barrier function, such as eczema. It is hypothesised that oral tolerance development is dependent on initial exposure of food antigens via the gastrointestinal tract and with eczema food antigens instead bypass the oral route and penetrate the disrupted skin barrier and are taken up by Langerhans cells, stimulating a TH2 response and the production of antigen-specific IgE antibodies and the development of food allergy.[114] Therefore, development of food allergy depends on the timing and balance between cutaneous and oral exposure.

Observational and randomised controlled trials have shown that the early introduction of allergenic foods into an infant's diet is associated with the reduced risk of food allergy, demonstrating that this is an important step in inducing oral tolerance.[22,115,116] Timing of solids

as well as prevention of eczema are central to this theory with studies also exploring the prophylactic use of emollients for the prevention of eczema and allergic sensitisation. Early studies have shown that emollient treatment is associated with a trend towards reduced incidence of eczema and food sensitisation.[117-119]

2.2 Tree Nut Allergy

Tree nut and peanut allergies share many clinical similarities in the severity of reactions and their lifelong nature and are also reported to commonly co-exist.[120]However, despite the potential severity of both peanut and tree nut allergy much of the literature to date has focused on peanut allergy epidemiology while tree nut allergy prevalence, natural history and adverse events have been under reported. There is very limited data on the prevalence of allergies to the individual tree nuts and factors related to the development of tree nut allergy.

The paucity of studies of tree nut allergy are likely due to the fact that previous allergy management advice for patients diagnosed with peanut and/or a single tree nut allergy was to avoid all nuts regardless of whether they were clinically allergic. This stemmed from assumptions regarding taxonomy, that tree nuts from similar families would have similar allergic capabilities and the high level of cross reactivity [6]reported between peanuts and tree nuts. The clinical dilemma is that these cross reactivity relationships do not always result in co-sensitisation on allergy testing or actual allergic reactions. Practical considerations also impacted advice to avoid all nuts. These considerations included potential difficulty for patients to distinguish one nut from another,[7,8] the potential cross contamination of nuts in food processing and the often lengthy and time consuming process of having to perform individual nut food challenges. It was also hypothesised that progression to further nut allergies could be prevented through blanket nut avoidance.[121]

However, there has been a paradigm shift in clinical practice with prevention strategies for food allergy around delaying the introduction of common allergens failing to show a benefit and observational studies[30,116,122] and a recent randomised controlled trial looking at the timing of introduction of peanut in high risk infants demonstrating that earlier introduction is protective.[22] Additionally, tree nuts have become an increasingly common part of our diet and there is increasing acknowledgement of the cardiovascular health benefits of consuming nuts.[10] It is also postulated that inclusion in the diet of non-allergic nuts improves the quality of life of those with nut allergies through less restrictive dietary avoidance.[123-125]The

culmination of this is clinical management of peanut and tree nut allergy is evolving and we can no longer base our assumptions of tree nut allergy on what we know of peanut allergy.

2.2.1 What is tree nut allergy?

To date, there is no standardised definition of what constitutes a nut. According to the botanical definition, nuts are a particular type of fruit, however, in culinary terms the term is used more broadly to include any large, oily kernels found within a shell and used in food.[126] The culinary nuts most likely to result in IgE-mediated allergic reactions are peanut and what is collectively referred to as tree nuts. Botanically peanuts are a legume, however we consume them like a nut. What constitutes a tree nut however, varies from country to country. For labelling purposes, pinenuts are considered a seed in Europe, but as a nut in North America. In the U.S. several additional foods are considered tree nuts including beechnut, butternut, chestnut, chinquapin, coconut, gingo, hickory nut, lychees and shea nut.[126]In Australia, the most common definition of tree nut and that adopted by Food Standards Australia New Zealand (FSANZ) includes the culinary nuts: almond, Brazil nut, cashew, hazelnut, macadamia, pecan, pistachio and walnut.[127] This is the tree nut definition that will be used throughout this thesis.

2.2.2 Tree nut allergenic proteins

Tree nuts have two major types of proteins, metabolic and storage proteins.[128]To date, 38 of these tree nut proteins have been shown to be reactive with IgE from allergic patients.[55] The seed storage proteins are the allergens associated with many cases of severe, anaphylactic tree nut allergies and include the prolamin superfamily (the 2S albumins) and the cupin seed globulins 7s and 11s[129] Other allergens known as the pan-allergens are similar to proteins in pollens, seeds, fruits and vegetables and are associated with IgE-mediated cross reactivity and include pathogenesis related proteins, PR-10 and the non-specific lipid transfer proteins (LTP).[130] A final group of recently identified allergens are the oleosins, which stabilise oil bodies in the seed. Among the tree nuts, oleosin has only been identified as an allergen in hazelnut.[131,132]A summary of the tree nut proteins identified to date is outline in Table 2.

Considerable research has gone into determining the biochemical and immunological characteristics of identified tree nut proteins. This has led to improved understanding of which

particular proteins are most strongly associated with clinical reactivity. Knowing which particular proteins are reacting with IgE may prove to be of diagnostic use in identifying those patients who have more severe and/or persistent food allergies. It has also allowed for greater understanding of the cross reactivity relationships between various nut proteins, with other food and inhaled allergens and analysis of effects such as processing, denaturation and the food matrix on allergenicity.[129]

2.2.3 Types of allergic reactions to tree nuts

There are two types of allergic reactions that can occur to tree nut proteins. Firstly the more immediate and severe IgE-mediated allergic reactions and secondly a milder form of IgE-mediated reaction known as oral allergy syndrome (OAS) or pollen food syndrome (PFS).

OAS/PFS is a type of food allergy where botanical plant family relationships and cross reactivity relationships are important and translate into clinical allergic reactions.[133]OAS/PFS occurs mainly in adolescents and adults with allergic rhinitis who are sensitised to particular inhalant allergens such as pollen, particularly birch pollen (Bet v1).[134]Foods within the same botanical family as the inhalant allergen can cross-react resulting in a localised oral allergic response. Symptoms consist of itching, burning, tingling and sometimes angioedema of the lips, tongue, roof of the mouth and throat. Cross-reactivity was once thought to be limited to the heat and gastric-labile PR-10 proteins within fruits, vegetables and nuts and explained the absence of gastrointestinal or systemic reactions and individuals being able to tolerate cooked forms of the foods. Labile profilins, or relatively heat stable lipid transfer proteins, have now been found to be cross-reactive and it is reported that 8.7% of OAS/PFS patients may experience co-existing gastrointestinal symptoms and 1.7% systemic reactions, including anaphylaxis.[135]

OAS/PFS is more prevalent in countries and regions where birch trees are commonly present, such as northern Europe, where up to 40% of patients with pollen allergy are said to be affected.[135] Australian reports of OAS/PFS are limited but range from 5-23%.[136,137]

OAS/PFS reactions to tree nut will not be explored as part of this thesis.

Table 3: Details of tree nut allergens identified to date.

	PR-10 Protein MW (kD)	**Profilin** MW (kD)	**Ribosomal Protein** MW (kD)	**Nonspecific lipid transfer protein LTP** MW (kD)	**11s globulin** MW (kD)	**7s globulin** MW (kD)	**Oleosin** MW (kD)	**2S Albumin** MW (kD)	**Isoflavone reductase homologe** MW (kD)	**Luminal binding protein** MW (kD)	**Vicilin-like protein** MW (kD)	**Manganese superoxide dismutase** MW (kD)	**Legumin** MW (kD)
Almond		Pru du 4 (14)	Pru du 5 (10)	Pru du 3 (9)	Pru du 6 (41)								
Brazil Nut					Ber e 2 (29)			Ber e 1 (9)					
Cashew					Ana o 2 (55)	Ana o 1 (50)		Ana o 3 (14)					
Hazelnut	Cor a 1 (17)	Cor a 2 (14)		Cor a 8 (9)	Cor a 9 (40)	Cor a 11 (48)	Cor a 12, Cor a 13 (14-17)	Cor a 14 (15-16)	Cor a 6 (35)	Cor a 10 (70)			
Macadamia	A 17kDa protein described by Sutherland, 1999.but not formally characterised to date.[138].												
Pecan					Car i 4 (55)			Car i 1 (16)			Car a 2 (55)		
Pistachio					Pis v 2 (32) Pis v 5 (36)	Pis v 3 (55)		Pis v 1 (7)				Pis v 4 (25-27)	
Walnut (Black)						Jug n 2		Jug n 1					Jug n 4 (34,22)
Walnut (English)		Jug r 5 Jug r 7 (13)		Jug r 3 (9) Jug r 8 (9)	Jug r 4 (58)	Jug r 2 (44)		Jug r 1 (14)			Jug r 6 (47)		

Source: www.allergen.org [55]

2.2.4 Tree nut allergy prevalence

Peanut allergy has been well described and widely reported with population prevalence estimates between 1-6%.[2,4] Despite the similarities to peanut allergy, the population prevalence of tree nut allergy has been less well characterised. Determining tree nut allergy prevalence at a population level can be complex. As described previously the definition of a tree nut can vary with regional variations common. Some studies include peanut and tree nuts together as "nuts", while other studies only include one or two tree nuts. Few studies investigate allergy to all eight common individual tree nuts. Secondly, as discussed in section 2.2.3 allergic reactions to tree nuts can result from primary IgE-mediated mechanisms or alternatively, via secondary cross reactivity mechanisms to birch pollen known as Oral Allergy Syndrome (OAS) or Pollen-Food-Syndrome (PFS). Finally, the method of tree nut allergy diagnosis may vary from self-reported methods such as surveys and questionnaires (which have been found to overestimate the true prevalence of food allergy)[139],IgE testing (which are limited to IgE-mediated food allergy and are indicative of sensitisation not clinical allergy), to the most objective but time consuming and cumbersome methods of OFC and double-blind-placebo-controlled food challenges (DBPCFC).

One previous systematic review of studies published between 1990-2006 by Zuidmeer et al, reported the prevalence of perceived reactions to tree nut as ranging between 0-7.3%. However, most studies included in this review (n=27 of 36) were based in Europe where the prevalence of OAS/PFS is high, and few studies used objective definitions of tree nut allergy such as challenge confirmed outcomes.[140] A more recent systematic review by Nwaru and colleagues reported a pooled prevalence estimate of tree nut allergy of 0.5% (95%CI 0.08-0.8) but was confined to European studies only and did not distinguish between individual tree nuts[3]. A large randomised, cross-sectional survey of 38,480 household in the U.S. between 2009-2010 reported parent-reported tree nut allergy prevalence among children of 1.0% (95%CI 0.9-1.2) and again did not distinguish between individual tree nuts. [13]

There is limited evidence to determine if the population prevalence of tree nut allergy is increasing. The only longitudinal data to date on tree nut allergy prevalence is limited to three studies in the United States that utilised random-digit telephone surveys in 1997, 2002, and 2008.[141-143] Study design was consistent across each sampling period and included a large number of participants (n=4374; 13,493 and 5300 respectively), however data was limited to

self-reported tree nut allergy. No significant increase in adult self-reported tree nut allergy prevalence was found over the three time points. However, the prevalence of self-reported tree nut allergy in children younger than 18 years had increased significantly (0.2% in 1997, 0.5% in 2002 and 1.1% in 2008). Proportionally, the increase was greater than that observed for peanut over the same time periods (0.4% in 1997, 0.8% in 2002 and 1.4% in 2008). Using emergency department visits as a proxy for increasing food allergy prevalence Dyer et al reported a 32% annual increase in emergency department visits for tree nut anaphylaxis in children over a 4 year period (2008-2012) in the U.S. state of Illinois. This observation may be a result of increased severity of tree nut reactions and therefore resulting in hospital presentation compared to other food allergens or a reflection of the increased usage of tree nut in our diets over this time period, however tree nut anaphylaxis was the highest increase in food-induced anaphylaxis reported in this study.[144] To date there is no data on changes in tree nut allergy prevalence over time in Australia and it will not be studied as part of this project.

My aims are to:

1. Review and summarise the worldwide prevalence of tree nut allergy.
2. Determine if tree nut allergy varies by region.
3. Identify the most common individual tree nut allergies around the world.
4. Determine the prevalence of tree nut allergy in Australian children.
5. Determine the most common tree nut allergies in Australian children.

This work will provide the first population-based, challenge-confirmed estimates for overall tree nut allergy and individual tree nut allergy in Australia.

2.2.5 Adverse events related to tree nut allergy

Adverse food reactions are common and hospital based admissions for food induced anaphylaxis are increasing in the UK and Australia.[145-147] However, reassuringly fatalities are rare with a systematic review in 2013 calculating that food allergic people would be at least 10 times more likely to die in an accident than of fatal anaphylaxis.[148] Food-induced anaphylaxis fatality rates have been reported as stable in the UK despite increasing admission rates for anaphylaxis[147]but fatalities have reportedly increased in Australia.[149,150] Peanut and tree nuts have been reported as the most common trigger of adverse food reactions and fatalities.[147,151-

[153] The challenge with food induced anaphylaxis is the unpredictability of severe reactions and there are currently no clinical or biochemical tests that can help clinicians predict which food-allergic patient is likely to develop anaphylaxis.[154] Current allergy tests can predict the likelihood of reaction, but not the severity.[31]

Tree nut anaphylaxis fatalities

Food induced anaphylaxis fatalities are rare with a recent systematic review and meta-analysis reporting an incidence rate in children (0-19 years) of 3.25 per million person-years (1.73, 6.10; range 0.94, 15.75; sensitivity analysis 1.18–6.13).[148] Case reports from the United States and the United Kingdom Fatal Anaphylaxis Registry (UKFAR) show that peanut and tree nuts account for 70-90% of reported food-induced anaphylaxis fatalities, with tree nuts accounting for around 18-40%.[147,155-158]

Although there is no formal reporting system for anaphylaxis fatalities in Australia, the patient advocacy and support group Allergy & Anaphylaxis Australia claim that between 2005-2016 there have been 14 cases of food-induced anaphylaxis deaths in Australia with five of those attributable to tree nuts (one of each for macadamia, pistachio and hazelnut and 2 due to walnut).[159] Published Australian data in 2016 reported 4 of 6 anaphylaxis fatalities in those <20 years of age were due to peanut or tree nuts between 1997-2013.[149] A summary of worldwide case reports for food induced anaphylaxis are outlined in Table 3.

Factors associated with increased risk of fatal anaphylaxis include the following:

a) Delayed or no administration of adrenaline during an episode of anaphylaxis.[149,155,156]
b) Age: the teenage and young adult age group (12-25 years old) constitutes the highest proportion of deaths from anaphylaxis (70-85%). [156]
c) Peanut or tree nut allergy with 70-90% of deaths from food-induced anaphylaxis due to these foods.[155-157,160]
d) Co-existing and poorly controlled asthma.[149,161]

It is important to note however, that factors that increase the likelihood of death from anaphylaxis have never been formally explored in a population setting and are limited to retrospective assessments of case series of fatalities and as such conclusions must be drawn

with caution. Exploration of risk factors for tree nut allergy fatalities will not be part of this thesis.

Table 4. Details of food induced anaphylaxis fatality case series

Country	Year	Total Number	Food	Source
USA	1994-2006	32	Peanut 37 Tree Nuts 18 Walnut =4 Brazil =2 Pecan =2 Pistachio = 1 Almond = 1 Hazelnut =1 Unknown = 7 Milk 5 Fish 2 Unknown 1	Bock, JACI 2001 107:191-3[155]
UK	1998-2012	124	Peanut 25 Tree nuts 15 Unknown nut 26 Fish and Crustacea 12 Milk 12 Unknown food 34	Pumphrey, *CEA, 2000*;30:1144-1150.[157] Pumphrey, JACI 2007;119(4):1018-1019.[158] Turner, JACI, 2015;135(4):956-963 e951.[147]
Japan	1999-2004	4	Shrimp 1 Buckwheat 1 Fish 1 Chocolate 1	Ebisawa M. In: James J, M, Burks AW, Eigenmann PA, eds. *Food Allergy.* Vol 1. Elsevier Saunders; 2012:113-126.[162]
Australia	1997-2016	22	Peanut 4 Tree nuts 4 Pistachio 1 Walnut 2 Macadamia 1 Hazelnut 1 Fish 1 Shellfish 2 Milk 6 Unknown 5	Mullins, CEA. 2016;46(8):1099-1110. Liew, JACI 2009;123(2):434-442[149,150] Unpublished information from Allergy & Anaphylaxis Australia.[159]

Tree nut induced allergic reactions

A recent systematic review and meta-analysis of food-induced anaphylaxis in patients with food allergy reported a pooled incidence rate of self-reported anaphylaxis in 0- to 19-year-olds of 4.93/100 person years (95% CI, 2.78-8.74) with a range of 0.6 to 8.9/100 person years based on 10 studies.[163] However, studies included were limited to selected food allergens, in younger children, or based on allergy clinic or health care facility presentation. Few population studies have described the frequency of adverse food reactions, including anaphylaxis. Although clinical samples have the strength of more robust diagnosis and definition of adverse reactions and anaphylaxis, they miss capturing those who do not present for medical care and are likely

to over represent those with more severe food allergy phenotypes, higher socioeconomic status, and greater access to specialised medical management and education.

Collectively peanut and tree nuts are the most common food trigger of anaphylaxis for emergency department presentations and hospital admissions.[144] Research over the past 25 years has estimated the annual incidence rate of accidental exposure in children with peanut and/or tree nut allergies in the U.K, the U.S and Canada to range widely, between 3-55% (Table 4). Estimate variations are likely a reflection of differences in study designs, age of participants and level of allergen avoidance education provided. To date, estimates are not available from population cohorts, nor is there Australian data.

My aims would therefore be to explore the frequency, type and severity of reactions that occur to tree nuts in the population-based SchoolNuts cohort compared to other types of food allergy and determine if more reactions and/or more severe reactions are occurring to tree nuts as has been previously reported in the literature in retrospective case series reports based on clinical cohorts.

Table 5. Published studies of frequency of adverse food reactions among those with nut allergies, stratified by country

Study	Country	Study Design	Sample Size	Population	Frequency of adverse reactions	Ref
Bock et al (1989)	U.S	Retrospective 1973-1985	46	Peanut allergic children, 2-14years (DBPCFC)	34.7% reported reaction in the past 12 months	[164]
Vander Leek et al (2000)	U.S	Prospective longitudinal	83	Peanut allergic children (0.4-6.8 years) with a (convincing history of peanut allergy and positive allergy testing)	Annual incidence rate of 33%	[165]
Neuman-Sunshine et al (2012)	U.S	Retrospective 1990-2009	782	Peanut allergic, 1-19years (convincing history of peanut allergy and positive allergy testing)	Annual incidence rate of 7.3% (95%CI 6.5-8.1)	[166]
Hourihane et al (1997)	UK	Retrospective	622	Peanut allergic, all ages (self-reported)	50% reported allergic reaction in the past 12 months	[167]
Ewan et al (2005)	UK	Prospective	615	Peanut and tree nut allergic children, 10months -15 years (Definition of allergy unknown)	Annual incidence rate of 55%	[168]
Clark et al (2008)	U.K	Prospective	785	Peanut and tree nut allergic children undergoing an allergy management program	Annual incidence rate of 3.0%	[169]
Cherkaoui et al (2015)	Canada	2004-2014	1941	Peanut allergic children (physician diagnosed)	Annual incidence rate of 12.4% (95%CI 11.4-13.4)	[170]

2.2.6 Development and natural history of tree nut allergy

For the majority of people diagnosed with peanut or tree nut allergies their allergies will not resolve. Peanut allergy natural history has been retrospectively reported on selected clinic populations[171-175] and more recently prospectively at the population level and resolution is reported at around 20%.[176]

Tree nut allergy natural history

Tree nut allergy natural history is less well understood with around 10% resolution often reported. This figure, however is based on one study in a tertiary level allergy clinic in the US in children (3-21yrs).[177] Studies recruited from tertiary clinics are likely to over represent those with more severe allergic disease and not necessarily reflective of the spectrum of cases presenting at the population level and as a result may under estimate the resolution of allergic disease. Confirmation of tree nut allergy at baseline in this study was based on known history of a reaction to tree nut however, 63% of participants who were eligible for a follow up food challenge refused a formal OFC. Resolution of tree nut allergy will not be studied as part of this thesis.

Co-allergies to nuts

Allergies to peanut and tree nuts are reported as commonly co-existing as well as multiple tree nut allergies, however estimates in the literature vary considerably largely due to variations in allergy definitions and have been limited to tertiary allergy clinics which may over represent participants with multiple food allergies.

Invitro cross-reactivity between peanuts and tree nuts has been reported as high as 86%[178] and tree nut sensitisation among those with peanut allergy as high as 87%.[179] However, the coexistence of clinical peanut and tree nut allergy is lower with estimates based on self-report between 20 and 60% [122,142,180,181] and OFC confirmed outcomes much lower. A retrospective review from the United Kingdom showed that among a group of 94 patients with peanut allergy, 23% were found to be sensitised to one or more tree nuts yet only 7.4% were allergic.[182] A more recent retrospective review in the U.S reported a co-allergy rate of only 4% of those with peanut allergy and tree nut sensitisation, however it must be noted that many of

those undergoing tree nut OFC had low level sensitisation (SPT<3mm and/or sIgE <2KuA/L) and no history of tree nut ingestion.[183] A retrospective review in France reported a co-allergy rate to tree nut among those with peanut allergy significantly higher (43.2%), with hazelnut the most common tree nut allergy observed. However, northern France is an area with high rates of birch pollen allergy and the form of tree nut allergy was not defined as part of this study and is likely to include a high proportion of OAS/PFS.[179]

Studies of co-allergy among the individual tree nuts are limited but the same observation of higher levels of co-sensitisation and lower rates of clinical allergy reported for peanut and tree nut are reported, highlighting the limitation of sensitisation data alone and the need for OFC confirmed allergy outcomes.[121] The strongest cross reactivities among tree nuts appear to follow botanical family associations, for example walnut-pecan in the Juglandaceae family and cashew-pistachio in the Anacardiaceae family.[178,184] The NutCracker study, a single centre, prospective cohort study of 83 children with tree nut allergy in Israel reported two thirds of those with walnut and cashew allergy were also allergic to pecan and pistachio respectively, while all those with pecan and pistachio allergy were allergic to walnut and cashew, respectively.[185]. Andorf and colleagues, in a study of 60 selected multi-food allergic patients reported all those allergic to walnut had co-existing pecan allergy. Interestingly, they reported a uni-directionality of the co-allergies with only two thirds of those patients allergic to walnut and cashew allergic to pecan and pistachio respectively suggesting that some allergenic proteins are shared while others are unique to cashew and walnut resulting in mono-allergy.[186]

Although studies to date on nut co-allergies are based on retrospective allergy clinic data where significant selection bias may occur, it does suggest that OFC confirmed tree nut allergy among those with peanut allergy and the prevalence of multiple tree nut allergies may not be as high as commonly reported and may be influenced by region. Studies in unselected population-based cohorts based on objective food allergy definitions, such as OFC, inclusive of tree nut ingestion history are needed.

Development of tree nut allergy

Development of tree nut allergy in childhood is also understudied. The first presentation for children with food allergy is often via reactions to milk, peanut and/or egg in infancy and early sensitisation to tree nuts has been reported in 19% of those with food allergies by 2 years of age.[169] How this early sensitisation relates to the subsequent development of tree nut allergy is

uncertain and has not been studied at the population level nor systematically assessed using protocolised challenges. The Learning Early About Peanut Allergy (LEAP) trial demonstrated that in infants at high risk of developing peanut allergy (patients with egg allergy and/or eczema and a subgroup already peanut sensitised), regular peanut consumption before the age of 12 months reduced the risk of peanut allergy at 5 years by 81%. However, extension of this concept to other food allergies, such as tree nut allergy has not yet been explored.[22]The current clinical dilemma remains as to what should be done regarding tree nut allergy testing and introduction advice for those with either peanut allergy or other forms of food allergy in infancy.[120,187] There are currently no data on the primary or secondary prevention of tree nut allergy and clinicians are currently limited by the diagnostic testing available for tree nut allergy.

2.2.7 Diagnosis of tree nut allergy

As for other forms of IgE-mediated food allergy, the diagnosis of tree nut allergy is reliant on a detailed medical history in conjunction with IgE testing via sIgE or SPT.[188] The clinical dilemma for tree nut allergy diagnosis is that for many patients with an existing food allergy they have no prior history of ingestion or reaction to tree nuts and the challenge is how to safely guide the introduction of several individual tree nuts.

Allergy testing for tree nuts is limited to SPT and sIgE with other *in vitro* tests such as specific IgE to allergen components and basophil activation testing not available for all tree nuts or limited outside research settings. Therefore, current testing methods cannot differentiate between clinical cross-reactivity and co-allergy, versus serological cross-reactivity and co-sensitisation in a given patient. Given that there is a high degree of co-sensitisation among the various nuts, yet likely lower levels of co-allergy, OFC is the only way to reliably determine individual tree nut allergy status for any tree nuts to which a patient is sensitised. Patients either continue to follow multiple nut exclusion diets based on sensitisation status alone or have to undergo multiple tree nut challenges to determine the allergy status of each individual nut, which is expensive, labour intensive and can place patients at risk of severe reactions. Some centres have trialled food challenges incorporating low risk nuts in a combined or multiple nut challenge to more efficiently determine individual tree nut allergy status.[182,189,190]

Allergen components associated with clinical allergy to tree nuts have been studied for cashew, pistachio, Brazil nut and walnut and sIgE cut-offs predictive of allergy have been reported (Table 6).[191]To date the application of sIgE to individual tree nut proteins for tree nut allergy diagnosis is limited to research settings and some larger tertiary allergy centres.

Table 6. Tree nut allergen components associated with clinical allergy with a reported cut-off for specific IgE testing

Tree nut	Component associated with clinical allergy	Specific IgE cut-off (kU/L) (% PPV)	Reference
Cashew	Ana o 3	0.16 (97%) 0.20 (95%)	Savvatianos et al, JACI 2015[192] Lange et al, Allergy 2017[191]
Brazil nut	Ber e 1	0.25 (94%)	Rayes et al, CEA 2016[193]
Walnut	Jug r 1	0.1 (91%)	Blankestijn et al, JACI 2017[194]

Similarly, there are limited studies exploring the application of BAT to tree nut diagnosis, with hazelnut studied most extensively[195-197]and additional tree nuts limited to one study published in abstract form to date.[198]

An alternative in the absence of CRD and BAT for tree nut allergens is the use of 95% PPVs for sIgE and SPT to assist clinicians with the diagnosis of tree nut allergies or to determine allergy resolution and guide the decision of when to perform an OFC. PPVs for SPT have now been widely published for peanut, egg, milk and sesame in both clinical and population cohorts. However, 95% PPV data for tree nuts remains limited. Only one study has reported a 95% PPV for walnut sIgE of 18.5kUA/L, however data for other tree nuts could not be calculated due to small numbers.[178] Ho and colleagues have reported the only published 95% PPVs for SPT of 8mm for tree nuts from the allergy clinic at the Royal Children's Hospital, Melbourne between 1995 and 2005. Their results were limited to cashew, hazelnut and walnut and again calculation of 95% PPVs for other tree nuts was limited by small sample size.[38]

There is a paucity of data reporting the association between SPT responses and the risk of challenge confirmed tree nut allergy in clinical cohorts and to date, no reports in population

samples. One of the limitations is access to sufficient sample sizes utilising the same SPT methods for calculation of 95% PPVs for each of the individual tree nuts.

I will begin to begin to address this in the final study of this thesis by:

1. Determining the SPT wheal sizes that correlate with 95% PPV predictability of a positive oral food challenge for cashew in two allergy clinic cohorts and two population-based cohorts of children, and secondly
2. Determine if these thresholds differ when stratified by allergy clinic or general population cohorts, and finally
3. Determine if these thresholds differ when stratified by known risk factors including co-existing peanut allergy, coexisting other food allergies, coexisting atopy, previous reaction history, age and gender.

This study will be invaluable in informing clinical practice in the care of children with food allergy allowing for more accurate, timely and cost effective diagnosis of cashew nut allergy. It will be the first study to validate clinic derived 95% PPVs for cashew SPT with population based data and will build on previously published work by this group on the use of 95% PPVs as predictors of peanut, egg and sesame allergy in infants.

3. METHODS

This thesis explores the prevalence, elements of the natural history and development of tree nut allergy and the frequency of adverse events related to tree nut allergy primarily. It utilises data from two population-based studies: the HealthNuts study a population-based study of children recruited at 12 months of age with follow up to 6 years of age and secondly the SchoolNuts study a population-based study of 10 to 14 year olds both done in Melbourne, Australia. A final study was undertaken as part of this thesis investigating cashew nut oral food challenge outcomes in the HealthNuts and SchoolNuts cohorts and two allergy clinic cohorts based at the Royal Children's Hospital in Melbourne.

The methods for the HealthNuts and SchoolNuts studies will be described in detail in this chapter together with the methods for the final study investigating cashew oral food challenge outcomes.

3.1 The HealthNuts Study

3.1.1 Overview

The HealthNuts Study is the largest single-centre, population-based, longitudinal study of paediatric food allergy recruiting children at 12 months of age and followed up at 4 and 6 years of age. The primary aims of the HealthNuts study are to measure the prevalence of IgE-mediated food allergy at the population level and assess modifiable risk factors.

In brief, 5276 12-month-old infants were recruited from council-run immunisation centres in Melbourne, Australia, between August 2007 and September 2011. Infants were offered SPT to four common food allergens and any infant with detectable SPT (≥ 1mm) invited into the Royal Children's Hospital, Melbourne for an OFC to test for food allergy long with 200 randomly selected SPT negative infants as controls. When the participants turned 4 years of age, their parents were contacted and invited to be part of HealthNuts wave 2, which involved a parental

questionnaire for all participants and clinic assessment for children who underwent OFC at age 12-months or reported new food reactions since 12 months of age.

At age 6 years the entire cohort (n=5276) were invited to participate in questionnaire and SPT assessment. Questionnaires were mailed to all participants capturing demographic details, history of food allergy and new food reactions, common allergen exposure information, history of asthma/wheeze and eczema. All participants were invited for an allergy/health assessment which included SPT to a predetermined panel of 8 foods (milk, egg, peanut, wheat, sesame, cashew, almond, and hazelnut). Assessments were conducted either in the child's home or at the Royal Children's Hospital. Those with positive SPT (>1mm) or parent-reported reactions to foods (consistent with an IgE-mediated allergy) were invited for a clinic appointment with a specialist allergy nurse and OFC were conducted when indicated by a standardised protocol (Table 7).

The HealthNuts study has reported challenge-proven food allergy prevalence and natural history data for egg, peanut and sesame at 12 months and 4 years of age, however tree nut allergy details have not been collated, analysed and reported to date. This thesis will report parent-reported tree nut allergy prevalence at 12 months of age and OFC confirmed tree nut prevalence at 6 years of age. The development of tree nut allergy among those with food allergy at 12 months will be explored as well as co-allergy patterns among peanut and tree nuts at age 6 years. The resolution of tree nut allergy is not possible with the current HealthNuts study data as tree nut allergy OFC were not performed at 12 months of age.

3.1.2 HealthNuts study sampling frame

HealthNuts is essentially a birth cohort that was recruited at 12-months of age. This age was selected for two reasons, firstly, SPT in very young infants may be difficult to interpret, and hence 12-months of age was selected as the age for recruitment. Secondly, at that time of recruitment, allergy prevention guidelines recommended that parents did not introduce peanuts until after 12-months and as such it was considered unlikely infants would have ingested peanut by this age. This was to minimise reporting bias of environmental exposures that may have occurred if parents were aware of their child's food allergy status.[199]

The Australian Vaccination schedule recommends a series of vaccinations at 12-months of age which are administered either at council-run immunisation centres or by a general practitioner in their clinic. In 2007, 92% of Victorian infants received standard vaccinations at 12-months of age and 46% of those received their vaccinations at council-run immunisation sessions.[200] Initially, recruitment was conducted at 170 community immunisation centres across Greater Metropolitan Melbourne, although two-thirds of infant immunisations were conducted within 50 immunisations centres. Summary statistics of demographic characteristics were compared between the 50 most-visited centres and all 170 centres, and it was found that recruiting from the 50 centres maintained representativeness and diversity of sample so recruitment continued at the reduced sample of 50 immunisation centres.

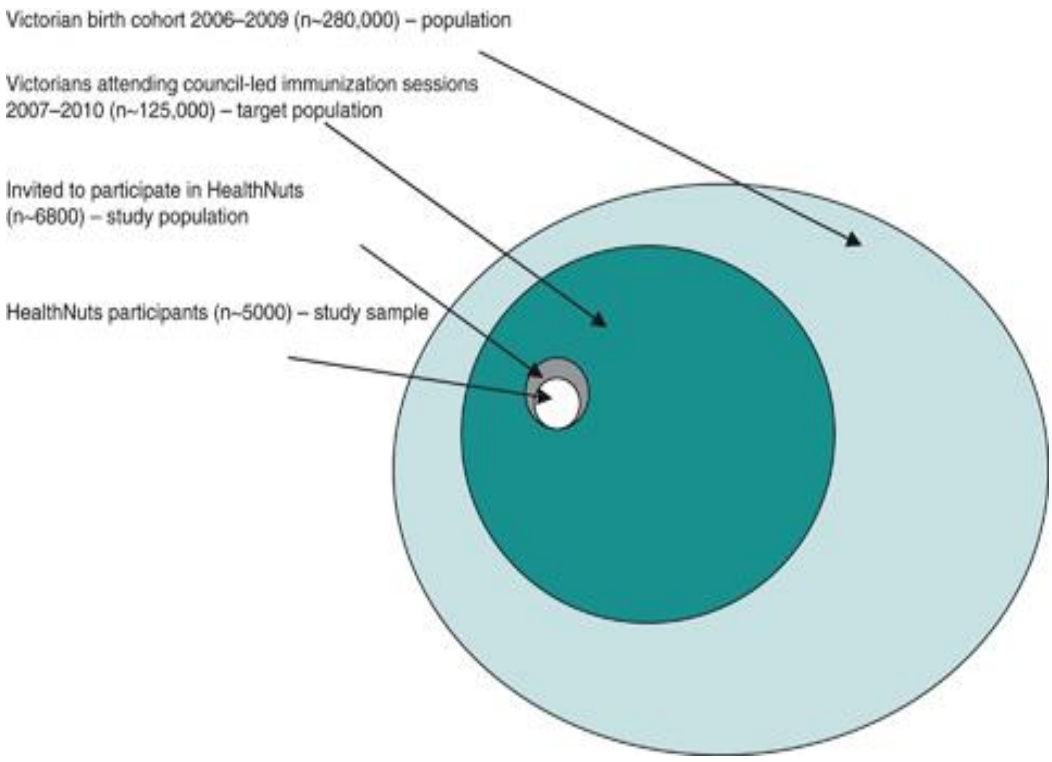


Figure 3.1. The HealthNuts study sample, representative of a Victorian birth cohort

Sourced with permission from Osborne et al, Clinical and Exp Allergy, 2010[199]

3.1.3 HealthNuts study recruitment

Age 1 year (Wave 1)

All parents of infants aged 11-15 months, attending selected immunisation centres for their 12-month-old immunisations between August 2007 and September 2011, were asked a series of scripted questions, inviting them to participate in a food allergy study. Infants were excluded from the study if their parents could not read or understand English.

During the compulsory 15-minute wait following immunisations, infants underwent skin prick testing to four common food allergens (peanut, egg, sesame/milk). Infants with any objective evidence of sensitisation, defined as SPT ≥ 1mm, were invited to participate in an OFC at the Royal Children's Hospital, Melbourne, Australia (83% of those eligible attended OFC). SPT was not conducted at recruitment if the infant had previously had an SPT performed by an allergist or if the infant had severe eczema on the back, which is a contraindication for SPT. These infants were still invited into the HealthNuts study clinic for food allergy assessment.

Parents completed a detailed questionnaire while SPT was undertaken, but prior to knowledge of the results. This was to reduce the potential for bias that may occur if parents were aware of their infant's sensitisation status and changed their reporting of exposures. The questionnaire captured information on infant's demographics, infant feeding, history of food reactions, common childhood illnesses, family history of allergy and environmental exposures (Appendix 1). These factors were identified on literature review and expert opinion as potential risk factors for food allergy. Details of tree nut ingestion and any adverse reactions were captured in this questionnaire.

Age 4 years (Wave 2)

At age 4 years, all participants were followed up via questionnaire (83% participation) and those who reported a new food allergy reaction consistent with IgE-mediated food allergy, and those who had any food allergy at age 1 year, were invited for clinic assessment which included skin prick testing to a predetermined panel of 8 foods (milk, egg, peanut, wheat, sesame, and the tree nuts - cashew, almond, and hazelnut) and OFC (Appendix 2). Those reporting new reactions to tree nuts since age one were offered SPT and OFC at Wave 2.

Age 6 years (Wave 3)

At age 6 years the entire cohort (n=5276) were invited to participate in questionnaire and SPT assessment. Questionnaires were mailed to all participants capturing demographic details, history of food allergy and new food reactions, common allergen exposure information, history of asthma/wheeze and eczema (Appendix 3). All participants were invited for an allergy/health assessment which included SPT to the same predetermined panel of 8 foods as age 4 years (60.5% participation) and was conducted either in the child's home or at the Royal Children's Hospital. Those with positive SPT (≥1mm) were invited for a clinic appointment with a specialist allergy nurse and OFC were conducted when indicated by a standardised protocol (Table 7).

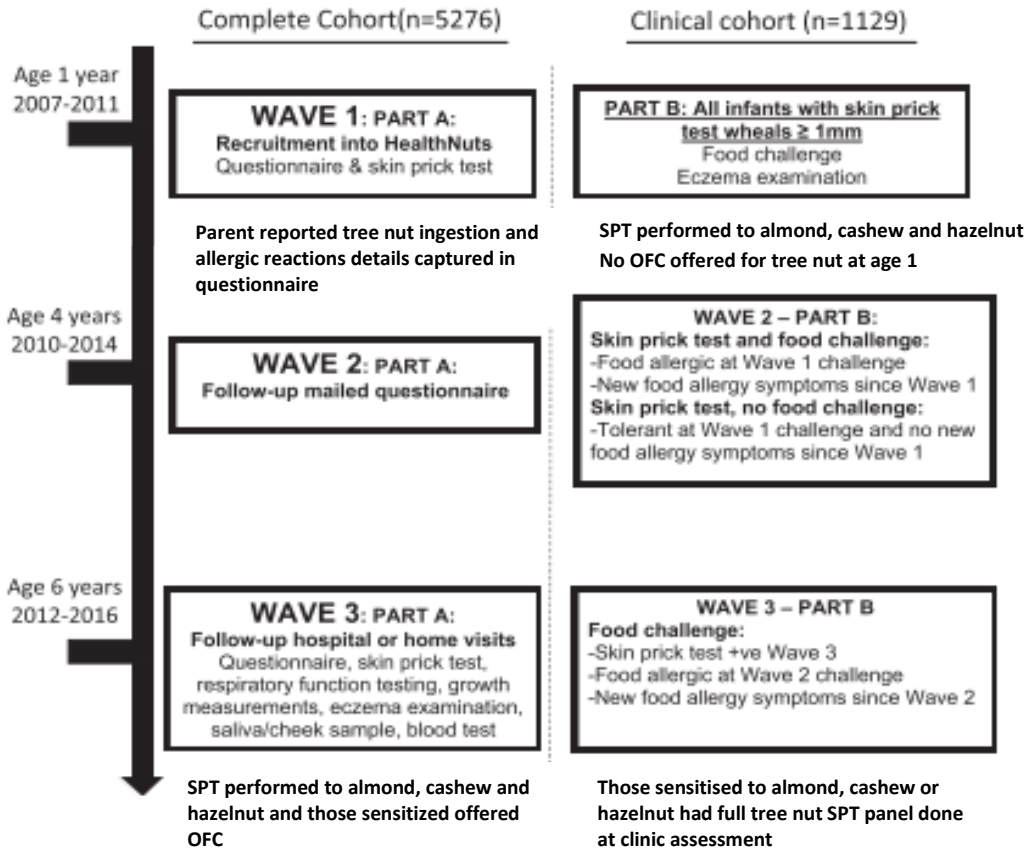


Figure 3.2. HealthNuts study overview and tree nut details available at each study wave

(Modified from Koplin et al. Cohort Profile: The HealthNuts Study: Population prevalence and environmental/genetic predictors of food allergy. Int J Epidemiol, 2015)[1]

3.1.4 HealthNuts study internal and external validity

To assess the internal validity of the HealthNuts study, a brief non-responder questionnaire was developed and administered to parents who declined to participate in the study (Appendix 4). The purpose of the non-responder questionnaire was to determine whether those who declined to participate in the study differed from those who participated with regards to whether the infant was eating and tolerating allergenic foods, family history of allergic disease, eczema and other known risk factors for food allergy.

The most common reasons for declining to participate in the HealthNuts study were that the infant was already eating and tolerating the foods being tested for (25%) or that they did not wish their child to undergo further testing following their immunisations (28%). Participants were similar to non-participants on most parameters measured in the non-responder questionnaire, with the exception that participants were more likely to have an immediate family member with a history of food allergy and less likely to have eaten peanut.[199]

To assess the external validity of the HealthNuts study relative to the target population of 12-month-old Victorian infants, characteristics of the HealthNuts cohort were compared to that of the whole population using data from the Perinatal Data Collection Unit, which publishes summary data from all Victorian births biennially.[201] For most characteristics, the HealthNuts sample was comparable to the general population, with the exception that older mothers were over-represented in the HealthNuts sample.[199]

3.1.5 HealthNuts study skin prick testing

Skin prick testing at age 1 recruitment (Wave 1)

During the compulsory 15-minute wait following immunisations, a nurse administered SPT on the infants back, using a single-time lancet (Stallergenes, Antony, France). The panel of allergens included four of five common food allergens, egg white, peanut, sesame and either shellfish or cow's milk (ALK, Spain), a positive control (10mg/ml histamine) and negative control (saline). Wheal size was measured after 15 minutes and calculated as the average of the longest diameter and the diameter perpendicular to it after subtracting the negative control.

Shellfish was initially included in the SPT panel, because the study failed to get ethics approval to conduct cow's milk SPT for fear that sensitisation rates would be high among infants already tolerating cow's milk and lead to unnecessary parental anxiety. This ruling was reversed in 2009 after a pilot study revealed very low rates of cow's milk sensitisation. After a sufficient number of shellfish SPT were conducted to calculate accurate prevalence rates (n~2000), cow's milk replaced shellfish in the SPT panel.

SPT to almond, cashew and hazelnut were performed only on those who attended RCH for a clinic assessment and OFC (n=1129).

Skin prick testing at age 6 follow up (Wave 3)

At age 6 years the entire cohort (n=5276) were invited to participate in questionnaire and SPT assessment (60.5% participation). SPT were performed on the child's back using the same methods as age one to a predetermined panel of 8 foods, including three tree nuts (milk, egg, peanut, wheat, sesame, and the tree nuts - cashew, almond, and hazelnut). SPT were conducted either in the child's home or at the Royal Children's Hospital.

3.1.6 HealthNuts study oral food challenges

Age 1 year oral food challenges (Wave 1)

Any infant with detectable SPT response, wheal size ≥ 1mm, was invited into the HealthNuts study clinic at the Royal Children's Hospital for an assessment and formal OFC to test for food allergy. This low SPT threshold was selected as a screening tool to ensure that no potential cases of food allergy were missed. A detailed clinical history was taken by an allergist to ensure that anaphylaxis to the index food had not occurred between recruitment and clinic attendance. SPT was repeated to a wider panel of allergens (peanut, egg white, sesame, shellfish, cow's milk, hazelnut, cashew, almond, soy, wheat and house dust mite.)

A decision tree was used to determine whether an OFC should proceed on the basis of previous exposure and reaction to the index food (Figure 3.3). Food challenges proceeded in all circumstances, with the exception of infants who had a definite exposure to the food and an unequivocal reaction, consistent with the pre-determined OFC stopping criteria in the previous 1-month for egg or 2-months for peanut and sesame.

Nurses performing OFC were blinded to both SPT results and clinical history. OFC were then performed irrespective of both the infants SPT wheal size and history of exposure to the food. OFC were conducted to peanut, raw egg white, baked egg and sesame, beginning with a smear or drop inside the lip. The dose increased at 15-20 minute intervals until either the equivalent of a single serve of the food was ingested, or a reaction occurred. After the initial dose, the challenge food is mixed with a previously tolerated food, usually apple sauce or yoghurt for the remainder of the challenge (Table 7).

Prior to the study commencing, investigators prospectively agreed on objective criteria that would constitute a positive OFC. These were:

- At least 3 concurrent, non-contact hives lasting at least 5 minute,
- Perioral or periorbital angioedema,
- Vomiting (excluding immediate gagging), or
- Evidence of anaphylaxis as defined by the Australian Society of Clinical Allergy and Immunology (evidence of circulatory or respiratory compromise) within 2 hours of the last challenge dose.

Infants were observed for 2 hours following the final challenge dose, or following an objective reaction that caused the OFC to cease. If there was a suspicion of an acute allergic reaction (e.g., a transient area of erythema or hives), that did not meet the criteria for a positive challenge, the next dose of the challenge protocol was administered. The dosage protocol for the OFC are detailed in Table 7.

At discharge, participants with a positive food challenge were educated about food allergen avoidance, provided with an appropriate action plan and advised to make a follow-up appointment in 1 years' time with an allergist. To capture late reactions, participants with a negative food challenge were requested to continue administering the top dose of the challenge food at home for 7 days and return a symptoms diary.

The food challenge result was deemed negative if the infant tolerated the top dose of the challenge and either did not report a late reaction after consumption of the top challenge dose at home for 1 week or if the infant's parent reported that the infant was regularly consuming and tolerating the food. Food challenges were deemed inconclusive and a repeat challenge

was offered if the infant refused to eat the challenge food at the clinic or if the parent reported a late reaction that did not meet the positive challenge criteria yet led the parent to remove the food from the infant's diet. Positive OFC in the absence of evidence of sensitisation were also considered inconclusive.

No OFCs were performed to tree nuts at age 1 year. Participants were advised to avoid any tree nuts that they were sensitised to as per standard clinical practice at the time (2007-2011).

At age 4 all those allergic at age 1 and those reporting new food reactions since age 1 were invited for allergy clinic assessment. Those reporting tree nut reactions consistent with IgE-mediated food allergy had SPT and OFC at age 4 years. At age 6 years all participants who were sensitised at follow up assessment were offered OFC including all tree nuts, refer to figure 3.3.

Age 1 negative food challenge controls

At 12 months a random sample of infants with negative SPT (0mm) to all foods at community recruitment in the HealthNuts study were also invited to undergo food challenge to egg or peanut. This was to test the assumption that infants with negative SPT are highly unlikely to have IgE-mediated food allergy to that food. All negative controls tested negative on food challenge.

Figure 3.3. Decision tree for oral food challenge at Age 1, HealthNuts Study

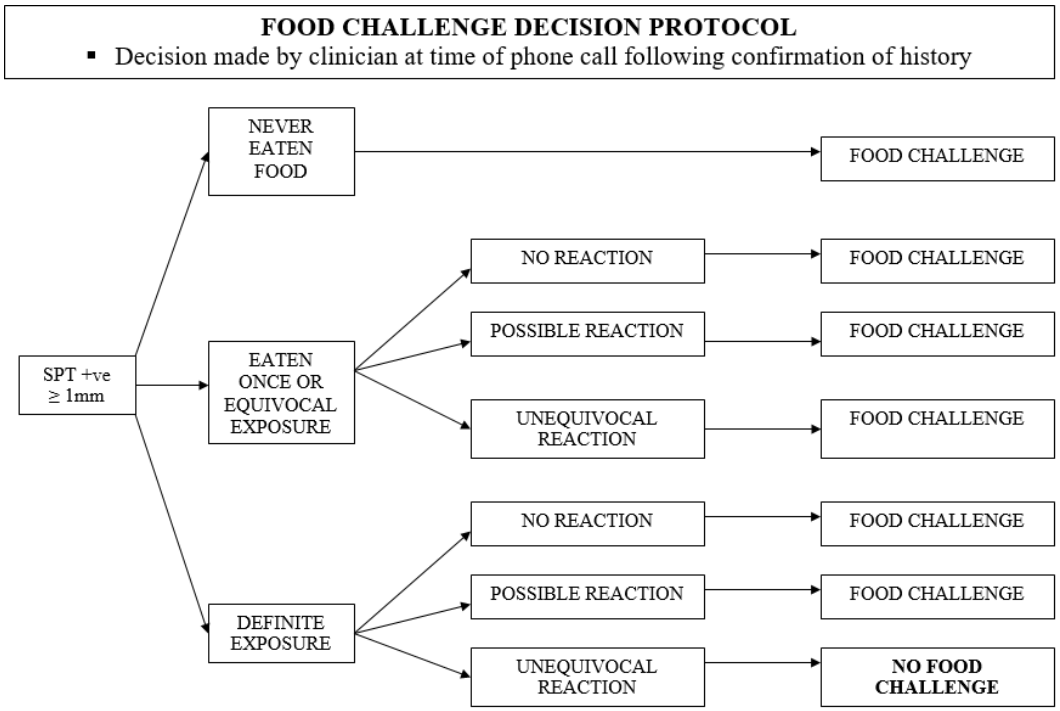
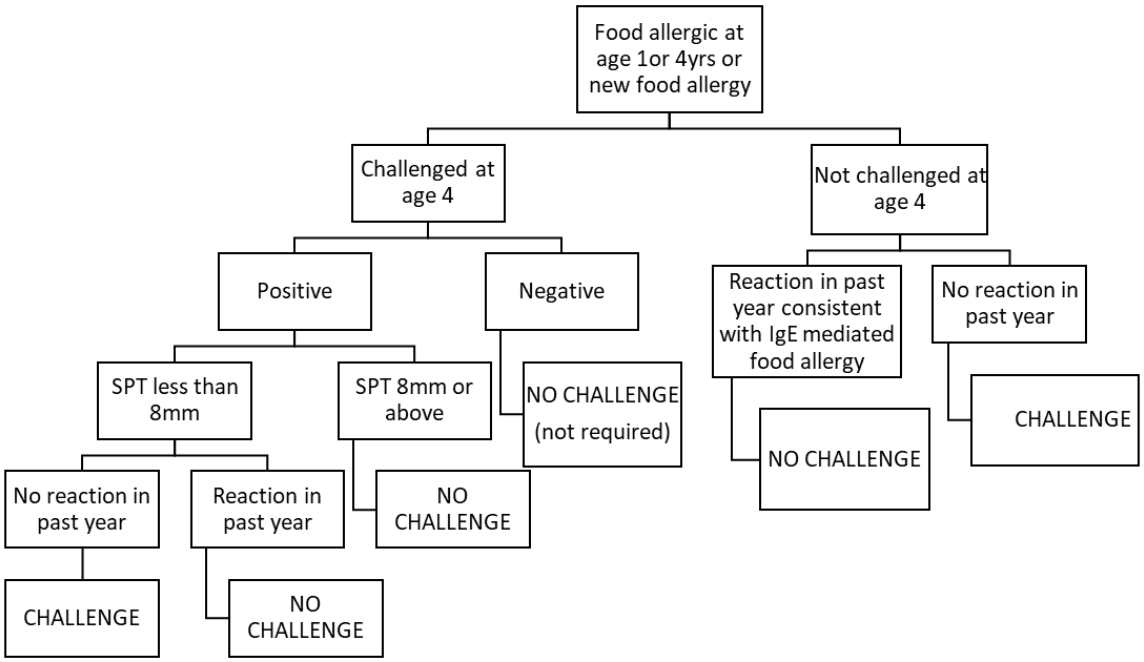


Figure 3.4. Flow chart of Age 6 years HealthNuts challenge criteria protocol



3.2 The SchoolNuts Study

3.2.1 Overview

The SchoolNuts Study is a large population-based study of students aged 10 to 14 years. The primary aims of the SchoolNuts study are to measure the population prevalence of food allergy during the critical 'early adolescent' period, defined as age 10 to 14 years,[202] using the gold standard of OFC and to estimate the frequency and predictors of accidental adverse reactions to foods among those with food allergy.

In brief, the SchoolNuts study is a school-based, cross-sectional stratified cluster sample of 9636 primary and secondary school students (10 to 14 years) recruited in greater metropolitan Melbourne. The study had two stages of assessment based on questionnaire and clinic assessment. Participants reporting a history of suggestive of food allergy were identified via self-report on student or parent questionnaires (Stage 1). Families were contacted via telephone to clarify the food allergy history and students invited to attend for a clinic appointment at the Royal Children's Hospital, Melbourne (Stage 2) where SPT was performed to the 16 most common food allergens (peanut, sesame, soy, almond, cashew, hazelnut, egg, shellfish, cow's milk, wheat, Brazil nut, walnut, pistachio, pine nut, macadamia, pecan). OFC was offered to all participants who self-reported suspected IgE mediated food allergy and had evidence of IgE sensitisation via SPT on the day of clinic. Data for this thesis includes the self-reported tree nut allergy outcomes and adverse events reported from the stage 1 student questionnaire and the cashew challenge outcomes from the stage 2 clinic phase.

3.2.1 SchoolNuts study sampling frame

A school-based, cross-sectional stratified cluster sampling of primary and secondary school students in greater metropolitan Melbourne (population 3.6 million)[203] was used to recruit a sample of 10 to 14 year old children. Seventy percent of the population of the state of Victoria reside in Melbourne.[203] Schools were selected for recruitment for efficiency and cost effectiveness due to the ability to access large amounts of students in a single study introductory session. Also recruitment from schools provided a population-based sample rather than limited to those students managed as part of tertiary hospital allergy clinics allowing more accurate estimation of population prevalence of food allergy and frequency of

adverse food reactions. Schools were randomly selected to reflect the variation in socio-economic status throughout school districts, and included each of the Government, Catholic and Independent school sectors. Schools were eligible for inclusion if they were less than 80km from the central business district and had more than 20 students per year level. A list of schools was obtained from the 2010 Melbourne Street Directory, stratified by primary versus secondary schools and subdivided into Government, Catholic and Independent schools. Each school within those groups was then assigned a number, and a random number generator was used by an independent statistician to select schools to approach.[204] These 229 schools (representing 20,965 students) were approached to participate.

3.2.2 SchoolNuts study recruitment

A summary of the SchoolNuts study recruitment is outlined in figure 3.5. Each randomly selected school had an information letter sent to principals inviting their school to participate in the study. At each participating school, all students in Years 5, 6 (primary schools), 7 and 8 (secondary schools) were invited to take part. The choice to recruit at 10 to 14 years allowed for assessment of children across the transition from primary to secondary school and across the period of onset of puberty. This period also sees a significant shift from parent and teacher supervision towards greater student autonomy and independence.

Researchers visited the schools to distribute a self-administered questionnaire to parent-consented students (Student Questionnaire-Appendix 5). Their parents were also asked to complete a questionnaire (Parent Questionnaire-Appendix 6). To improve the parent participation rate, modified versions of the Parent Questionnaires with survey questions shortened were subsequently sent by mail, email or SMS to those who had not completed the full Parent Questionnaire.

3.2.3 SchoolNuts study internal and external validity

To assess the internal validity of the SchoolNuts study, schools who did not want to participate were contacted via telephone to complete a short questionnaire to determine the number of children recorded as being at risk of anaphylaxis in the school and their age and gender.

The proportion of participating schools in each of the government, Catholic, and independent school sectors reflected the overall distribution of school types in Victoria, with the exception of slightly fewer government primary schools.[205]There were no consistent patterns for differences in participation according to either size of the school or socio-educational advantage (Table 6). Socioeconomic status of participants was determined by using the Index of Community Socio-educational Advantage (ICSEA) score for each school, which is generated from student-level parent occupation and education, location, and percent indigenous student enrolment developed by the Australian Curriculum, Assessment and Reporting Authority. Participating government primary schools had a slightly lower median ICSEA score, which indicates less socio-educational advantage.

In 2011, 3.9 million people resided in greater metropolitan Melbourne which represented 70% of residents in the state of Victoria. The distribution of SchoolNuts characteristics, including distribution of school sector attendance was comparable to general population data for the state of Victoria, collated by the department of education and available as an annual report.[205]

3.2.1 SchoolNuts Questionnaires

The SchoolNuts study included both a student and parent questionnaire with variations in questions asked across the two questionnaires (Figure 3.5 and Appendices 5 and 6). The student questionnaire included questions regarding the child's history of food allergy and asthma and knowledge and attitudes toward food allergy. Students reporting current food allergy, current asthma or both were asked to complete additional more detailed sections of the questionnaire regarding their asthma, food allergy, or both. Those with current food allergy were asked to specify foods to which they were allergic: peanut, other nuts (nut type specified), hen's egg, cow's milk, sesame, fish, shellfish, soy, and other food (specified) and provide details of any allergic reactions that had occurred in the past 12 months (Appendix 5).

The Parent Questionnaire collected additional information on the student's history of food allergy along with family demographics, and the allergy history of the other family members. The parent questionnaire did not contain details of any food reactions in the past 12 months (Appendix 6).

Student eligibility for clinic evaluation was via a two-step process. Based on the assumption that parents have a better understanding about the history of the student's food allergy, students with possible current food allergy were identified through the response to Parent Questionnaire. Broad criteria were used to capture all cases of possible current food allergy, which was a positive response to any of the following questions;

1) "Does your child currently have food allergy?"

2) "Has your child ever had food allergy, a food reaction or food related anaphylaxis?"

3) "Has your child ever eaten the following common allergens (Egg, Cow's milk, Sesame, Fish, Shellfish, Soy, Peanut, Tree nuts)?" to capture students who may have unrecognised food allergy.

Trained allergy research nurses then collected further information of the reaction/allergy by phone to evaluate whether current food allergy was likely, and if so, whether it was possibly IgE-mediated or not. Students were invited for clinic evaluation when the history suggested current IgE-mediated food allergy (i.e. evidence of an acute allergic reaction following ingestion of a food).

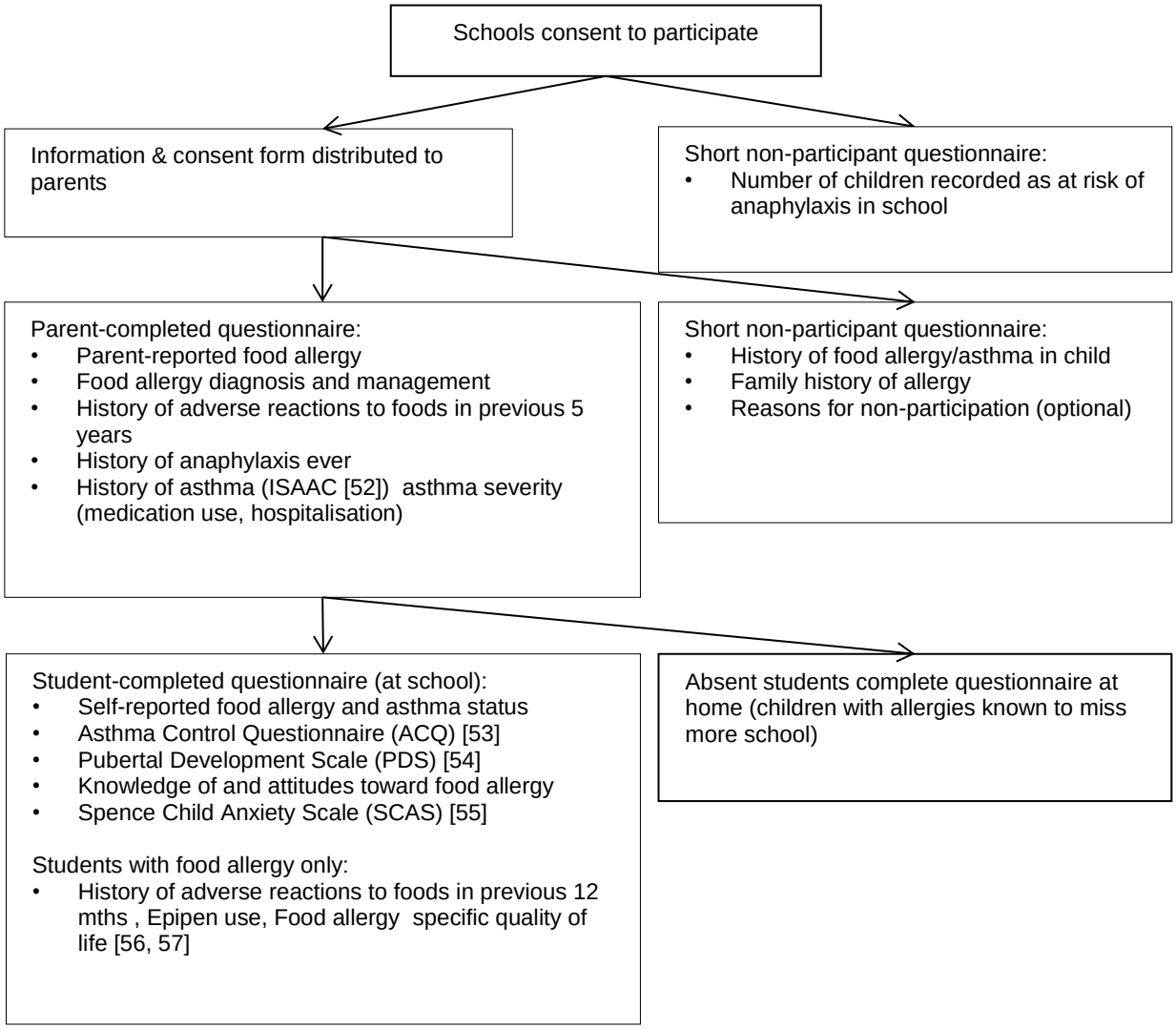
Data regarding adverse food reactions included in this thesis was based on the student questionnaires as this information was not included in the parent questionnaire. Student-reported food allergy was validated through comparison to parent questionnaire responses.

Table 7. Differences between participating schools and those that declined participation in the SchoolNuts study.

Characteristic	Participated	Declined	P value (Wilcoxon-Mann-Whitney test)
Government: primary	**N=31**	**N=22**	
ICSEA* (median, SD)	**1018(61.6)**	**1033(69.8)**	**0.03**
Size (no. students – median, SD)	**386 (188.0)**	**419 (210.8)**	**0.22**
Government: secondary	**N=19**	**N=25**	
ICSEA* (median, SD)	**977 (46.5)**	**968(53.0)**	**0.45**
Size (no. students – median, SD)	**736(491.8)**	**642 (346.5)**	**0.96**
Catholic: primary	**N=32**	**N=26**	
ICSEA* (median, SD)	**1055(56.3)**	**1078 (64.9)**	**0.17**
Size (no. students – median, SD)	**263 (118.2)**	**345 (152.9)**	**0.11**
Catholic: secondary	**N=8**	**N=18**	
ICSEA* (median, SD)	**1017(77.8)**	**1029 (56.1)**	**0.45**
Size (no. students – median, SD)	**890 (445.2)**	**778 (307.2)**	**0.87**
Independent: both	**N=15**	**N=11**	
ICSEA* (median, SD)	**1151 (51.3)**	**1152 (57.6)**	**0.42**
Size (no. students – median, SD)	**759 (483.2)**	**859 (640.6)**	**0.55**

Index of Community Socio-educational Advantage (ICSEA) is a scale of socio-educational advantage generated from student-level parent occupation and education, remoteness and percent indigenous student enrolment. Lower scores indicate more disadvantage.

Figure 3.5. Outline of SchoolNuts Study Part 1: Parent and student questionnaire phase



3.2.2 SchoolNuts skin prick testing

Students identified with parent-reported possible food allergy from Stage 1 underwent a skin prick test (SPT) to a panel of 15 food allergens (egg white, cow's milk, soy, peanut, cashew, almond, hazelnut, walnut, pistachio, macadamia, pecan, brazil nut, pine nut, sesame, shellfish) along with a positive and a negative saline control (ALK-Abelló SA, Madrid, Spain) as well as any other reported allergens using a single tine lancet (Stallergenes, Antony, France) on the student's volar forearm. Blood samples were also collected for serum IgE measurement.[204]

3.2.3 SchoolNuts oral food challenges

Students were eligible for OFC if they had a positive SPT result to a food they had a history of reaction to and currently avoiding, or they had never eaten. OFC were not performed if the participants met the following criteria consistent with a high likelihood of clinical allergy.

1. SPT ≥ 3mm AND one of more of the following:

a) A past history of severe reaction requiring multiple doses of adrenaline,
b) An episode of anaphylaxis when older than 10 years;
c) A convincing history of recent reaction (in the past 12 months) consistent with IgE-mediated food allergy;

2. A past history of reaction and highly sensitized (SPT wheal size ≥ 8mm).

A small number of OFCs were conducted despite a negative SPT due to equivocal history.

OFC dosage protocols were consistent with those of the HealthNuts study and included graded, incremental doses administered at 15 to 20 minute intervals (Table 7). Criteria to define a positive OFC result were based on the standardised criteria used in HealthNuts Study with one modification, namely, the inclusion of strictly defined subjective persistent symptoms in the upper airways or gastrointestinal which specifically included: itchy mouth or throat, abdominal pain or nausea, tightness in throat, difficulty talking or difficulty breathing, continuing up to the timing of the next dose, the previous dose was repeated. If the above symptoms persisted for a total of more than 40 minutes or reoccurred on 3 doses, it was recorded as a positive reaction.[204]

As per the HealthNuts study, OFCs were deemed negative when the student had a negative result on the day of the OFC and did not report any positive reactions during home-based food introduction in the week following the OFC.

Table 8. Oral Food Challenge Protocols for the HealthNuts and SchoolNuts Studies

Food	Peanut	Tree Nut	Raw egg white	Baked egg	Sesame
Source	Smooth peanut butter paste (Kraft, Port Melbourne, Australia)	Crushed whole tree nut	60-g free-range egg (Coles, Glen Iris, Victoria, Australia)	Vanilla cake mix (Green's Foods Limited, Glendenning, NSW, Australia)	Unhulled tahini (Mayver's, Altona North, Victoria, Australia)
Dose frequency	Every 20 minutes	Every 15 mins	Every 15 minutes	Every 15 minutes	Every 20 minutes
Dose 1	Smear inside lip	Smear inside lip	Drop inside lip	A ''crumb''	Drop inside lip
Dose 2	1/16 teaspoon	1/16 teaspoon	0.5 mL	1/12 muffin	0.31 mL
Dose 3	1/8 teaspoon	1/8 teaspoon	1 mL	1/6 muffin	0.62 mL
Dose 4	1/4 teaspoon	1/4 teaspoon	2 mL	1/4 muffin	1.25 mL
Dose 5	1/2 teaspoon	1/2 teaspoon	5 mL	1/2 muffin	2.5 mL
Dose 6	1 teaspoon	1 teaspoon	10 mL		5 mL
Dose 7	2 teaspoons	2 teaspoons	Remainder of egg white (10-13 mL)		-
	-	-			
Cumulative dose	1.94 teaspoons of peanut butter (11.3 g)		Raw white of 1 60g egg	10g whole egg baked at 180°C for 20 minutes	9.7 mL tahini (11.3 g)

Table 9. Tree nut protein amounts at OFC, top dose of 1 teaspoon

1 level 5ml teaspoon	Average total weight in 1 level teaspoon (grams)	Average total mg nut protein in 1 level teaspoon
Almond meal	4.0	1200
Hazelnut meal	4.0	600
Crushed walnut	2-2.5	550
Crushed cashew	2.5-3.0	700
Macadamia	3.0	300
Pecan	2.5	250
Pistachio	3.0	600

3.3 Cashew oral food challenge outcomes study

For the final study and manuscript on cashew oral food challenge outcomes and 95% positive predictive values for cashew SPT (chapter 4), data from 2 clinical cohorts were collected and compared to the population cohorts of the HealthNuts and SchoolNuts studies.

Clinic Cohorts

A retrospective analysis of all sequential open cashew OFCs conducted at the Royal Children's Hospital (RCH) allergy centre between 2011-2016 and a private allergy clinic based at RCH, the Melbourne Allergy and Children's Centre (MACCS) (2015-2016, operational since 2015) was performed by myself. Patients undergoing cashew OFC were identified from the RCH electronic medical record and a database at MACCS. Cashew SPT wheal size, co-existing food allergy, current and resolved comorbid allergic disease and food and environmental allergen sensitisation were extracted via chart review. Those with a cashew SPT >3 months prior to OFC were excluded.

Population Cohorts

Cashew OFC from the HealthNuts and SchoolNuts studies were included. As previously outlined, both studies recruited participants from the community and utilised the same SPT and OFC protocols. Cashew OFCs were performed at age 4 and 6 years in the HealthNuts study and age 10 to 14 years in the SchoolNuts study.

3.4 Statistical methods

Each manuscript in the subsequent results chapters includes a section describing the statistical methods specific to that analysis. As an overview, prevalence estimates are expressed as observed proportions with 95 % confidence intervals. Prevalence estimates were adjusted to reflect the distributions of risk factors among those approached at age 6 and agreed to follow up and therefore had a full allergy assessment versus those who declined follow up, via the inverse probability weighting method.[206]

Kappa analysis was performed to measure the agreement between dyads of student and parent responses for self-reported food allergy in the SchoolNuts study. All analyses used logistic regression (for binary outcomes) and linear regression (for continuously valued biomarkers to permit adjustment of the estimated difference in prevalence / mean for possible confounding of these associations by other measured factors). A separate regression model was conducted for each clinical factor with the demographic factors included as *a priori* confounders and other clinical factors only retained as confounding variables if they altered the odds ratio by more than 10%.

Receiver Operating Characteristic (ROC) curves were used to assess the diagnostic value of SPT and sIgE to predict food allergy and the area under the curve (AUC) was used to quantify the accuracy of the test (Chapter 4 and Chapter 6). The odds of food allergy (a monotonic transformation of the prevalence of food allergy) was modelled for various SPT or sIgE thresholds using logistic regression and predicted prevalence from these models replaced the observed values (presence or absence of food allergy) in the standard formula for the positive predictive values (PPVs). Bootstrapping, a method of deriving standard errors and confidence intervals from repeated samples drawn with replacement from the original dataset, was used to quantify the precision (uncertainty) of the PPV estimates and to calculate 95% confidence intervals for the corresponding population parameter.[207] For the thresholds with 95% PPV, the sensitivity, specificity, NPV, and positive and negative likelihood ratios were calculated using standard formulas the details of which are discussed in the literature review section 2.1.1.

STATA software was used for all statistical analysis (release 14.0 and 15.0, StatCorp, College Station, Texas).

3.5 Ethical conduct in human research

The subjects of these studies were infants and young children which raises several ethical considerations. The Declaration of Helsinki, a set of ethical principles for medical research, asserts that vulnerable populations such as infants and children, should not participate in medical research unless their involvement is essential to answer a specific research question that will promote the health of that vulnerable population. As food allergy generally presents in the first years of life and due to the paucity of methodologically robust food allergy studies,

it is important to conduct research in this age group to identify risk factors and predictors of food allergy and its natural history. Participation in this study included SPT screening for food allergy. It poses minimal risk to an infant and the benefits include identifying infants at risk of food allergy before introduction of the allergen.

Consent to participate in the HealthNuts study was provided by the infant's parents or guardians. Approval to conduct the HealthNuts study was obtained from the Victorian State Government Office for Children (reference no. CDF/07/492), the Victorian State Government Department of Human Services (reference no. 10/07), and the Royal Children's Hospital Human Research Ethics Committee (HREC number. 27047).

Consent to participate in the SchoolNuts study was provided by the student's parents. Ethical approval was obtained from the Royal Children's Hospital Human Research Ethics Committee (HREC number 31079), the Department of Education and Early Childhood and the Catholic Education Office.

Ethical approval for the cashew OFC project was obtained from the Royal Children's Hospital Human Research Ethics Committee (HREC number 37076A).

4. THE WORLDWIDE PREVALENCE OF TREE NUT ALLERGY

4.1 Introduction

This chapter is presented in the form of a manuscript that was published in Current Allergy and Asthma Reports. 2015 Sep; 15(9):555.

This was the first study done as part of this thesis and addresses the following research questions via a systematic review of the literature:

1. What is the worldwide prevalence of tree nut allergy?
2. Does tree nut allergy prevalence vary by region?
3. What are the most common individual tree nut allergies around the world?

This research was presented at the Food, Allergy and Nutrition Symposium hosted by the NHMRC funded Centre for Food & Allergy Research (CFAR) and the CRE in Foods for Future Australians in 2015. It was also presented as an abstract and poster at the Australian Society of Clinical Immunology and Allergy (ASCIA) annual scientific meeting in 2015 both held in Adelaide, Australia

4.2 The Manuscript

Curr Allergy Asthma Rep (2015) 15: 54
DOI 10.1007/s11882-015-0555-8

FOOD ALLERGY (T GREEN, SECTION EDITOR)

The Prevalence of Tree Nut Allergy: A Systematic Review

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Abstract Tree nuts are one of the most common foods causing acute allergic reactions and nearly all tree nuts have been associated with fatal allergic reactions. Despite their clinical importance, tree nut allergy epidemiology remains understudied and the prevalence of tree nut allergy in different regions of the world has not yet been well characterised. We aimed to systematically review the population prevalence of tree nut allergy in children and adults. We searched three electronic databases (OVID MEDLINE, EMBASE and PubMed) from January 1996 to December 2014. Eligible studies were categorised by age, region and method of assessment of tree nut allergy. Of the 36 studies identified most were in children (n=24) and from Europe (n=18), UK (n=8) or USA (n=5). Challenge-confirmed IgE-mediated tree nut allergy prevalence was less than 2 % (although only seven studies used this gold standard) while probable tree nut allergy prevalence ranged from 0.05 to 4.9 %. Prevalence estimates that included oral allergy syndrome (OAS) reactions to tree nut were significantly higher (8–11.4 %) and were predominantly from Europe. Prevalence of individual tree nut allergies varied significantly by region with hazelnut the most common tree nut allergy in Europe, walnut and cashew in the USA and Brazil nut, almond and walnut most commonly reported in the UK. Monitoring time trends of tree nut allergy prevalence (both overall and by individual nuts) as well as the prevalence of OAS should be considered given the context of the overall recent rise in IgE-mediated food allergy prevalence in the developed world.

Keywords Tree nut allergy · Systematic review · Prevalence · Epidemiology

Abbreviations

Primary tree nut allergy	IgE-mediated allergic reaction upon exposure to tree nuts that is due to a specific immune response directed against tree nut allergens
Secondary tree nut allergy	IgE-mediated allergic reaction upon exposure to tree nuts that is due to cross-reactivity of specific IgE directed against non-tree nut allergens
Tree nut sensitisation	Presence of tree nut allergen-specific IgE measured by skin prick test (SPT) or specific IgE blood testing (sIgE)
Oral allergy syndrome (OAS)	A secondary tree nut allergy that occurs predominantly in pollen-sensitised individuals, mediated by cross-reactive IgE responses to allergens present in pollen and other plants. Presents with oral pharyngeal symptoms (itching mouth/tongue)
Pollen food syndrome (PFS)	Another term for oral allergy syndrome

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Introduction

Tree nut is the collective term used to describe nuts that grow on trees. Contrary to popular belief, peanuts are not tree nuts and are in fact a groundnut and classified as a legume. Tree nuts most likely to result in an IgE-mediated food allergy reaction are almond, brazil nut, cashew nut, hazelnut, macadamia, pecan, pistachio and walnut. Although botanically unrelated, tree nut and peanut allergies share many clinical similarities. Peanut and tree nuts are two of the most common foods reported to cause IgE-mediated food allergic reactions. IgE-mediated food allergy reactions can occur after ingestion of very small amounts of peanut and tree nut, typically within minutes of ingestion with symptoms including hives, angioedema or vomiting. Reactions can also be life threatening, with the most severe reactions termed anaphylaxis. Peanut and tree nuts together account for 70–90 % of reported food-induced anaphylaxis fatalities, with tree nuts alone accounting for around 18–40 % [1–4]. Allergies to peanut and tree nuts also commonly co-exist with around 20–30 % of people with a peanut allergy also allergic to one or more tree nuts [5, 6]. For individuals with one tree nut allergy, around 30 % will have at least one additional tree nut allergy [6]. Tree nut and peanut allergies are usually lifelong [7]. Peanut allergy has been well described and widely reported with population prevalence estimates between 1 and 6 % [8•, 9]. Despite the similarities to peanut allergy, the population prevalence of tree nut allergy has been less well characterised.

Determining tree nut allergy prevalence at a population level can be complex. Firstly, the definition of a 'tree nut' may vary. Some studies include peanut and tree nuts together as 'nuts', while other studies only include one or two tree nuts. Few studies investigate allergy to all eight common individual tree nuts. Secondly, allergic reactions to tree nuts can result from primary IgE-mediated mechanisms or, alternatively, via secondary cross-reactivity mechanisms to birch pollen, in a form of food allergy known as oral allergy syndrome (OAS) or pollen food syndrome (PFS). In individuals with birch pollen sensitisation, birch pollen-specific IgE can cross-react with similar proteins found in a range of fresh fruits, vegetables and nuts (apple, apricot, carrot, celery, hazelnut, peach, peanut, pear, potato and plum) resulting in oral pharyngeal symptoms [10, 11]. Finally, the method of tree nut allergy diagnosis may vary from self-reported methods such as surveys and questionnaires (which have been found to overestimate the true prevalence of food allergy) [8•, 9], IgE testing methods such as skin prick testing (SPT) or specific IgE (which are limited to IgE-mediated food allergy and are indicative of sensitisation not clinical allergy), to the most objective but time-consuming and cumbersome methods of oral food challenge (OFC) and double-blind placebo-controlled food challenges (DBPCFC).

One previous systematic review by Zuidmeer et al., of studies published between 1990 and 2006, reported the prevalence of perceived reactions to tree nut as ranging between 0 and 7.3 %; however, most studies included in this review (n=27 of 36) were based in Europe where the prevalence of oral allergy syndrome is high, and few studies used objective definitions of tree nut allergy such as challenge confirmed outcomes [12•]. A more recent systematic review by Nwaru et al. was confined to European studies only and did not distinguish between individual tree nuts [13•]. A more up-to-date global prevalence estimate of tree nut allergy is needed since 20 new studies have been published since 2006, and understanding the regional variation in tree nut allergy is important given the overall rising burden of food allergy [14, 15] in developed countries and the importance of tree nuts as a cause of severe allergic reactions.

The aim of this paper is to provide a comprehensive, up to-date systematic review of the population prevalence of tree nut allergy in children and adults including details of all individual tree nuts in various regions of the world.

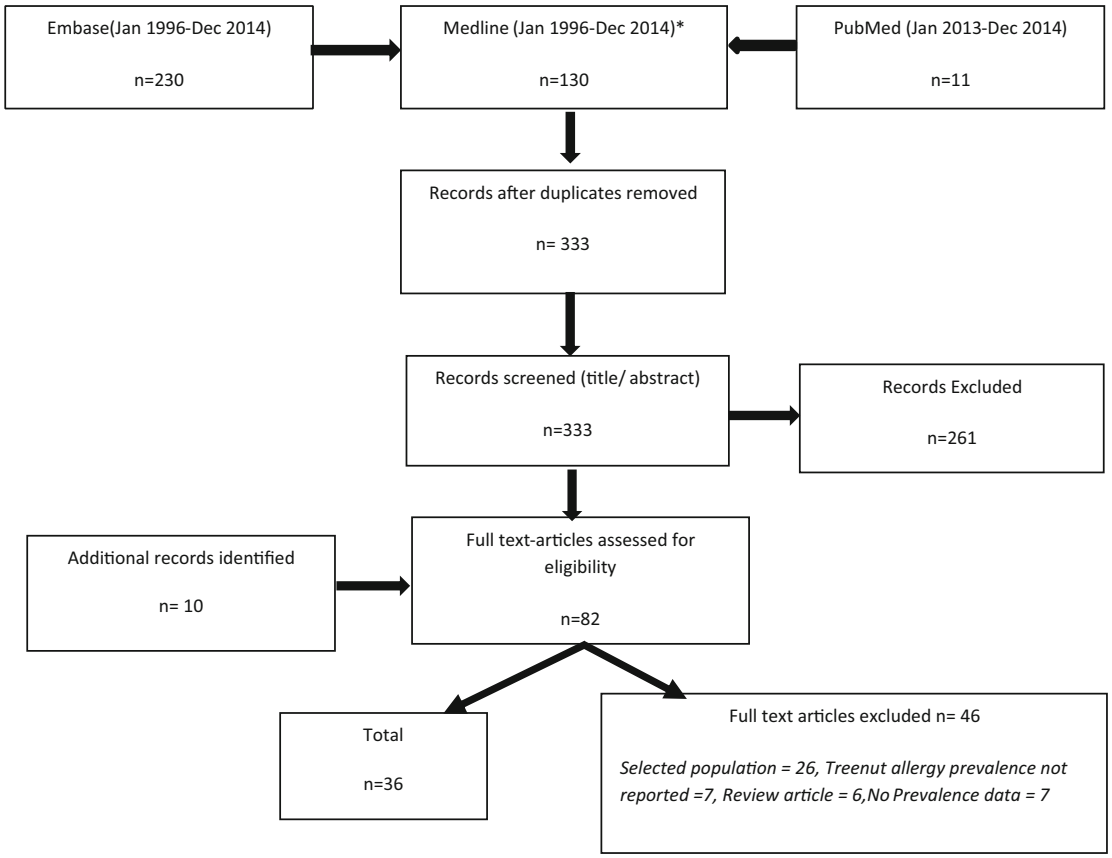
Methods

Search Strategy

Following closely the methods and procedures of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [16], we systematically searched three electronic databases (OVID MEDLINE, EMBASE and PubMed) based on a search strategy formulated with the assistance of a research librarian. The search strategy was created in OVID MEDLINE and modified for EMBASE and PubMed. Figure 1 outlines the full OVID MEDLINE search strategy.

Study Selection

Tree nuts were defined as walnut, almond, pistachio, cashew, pecan, hazelnut, macadamia and Brazil nut. Studies reporting on all forms of allergic reactions (primary and secondary IgE-mediated and non-IgE-mediated reactions) were included and there were no age restrictions applied. All tree nut allergy outcomes were included for both individual and combined tree nut allergies. We included eligible studies that reported tree nut allergy based on self-report, sensitisation (sIgE or SPT), OFC/DBPCFC or convincing clinical history. The search was limited to English-language articles and, to capture more recent publications, limited to the period January 1996 to December 2014. To ensure unbiased estimates of tree nut allergy prevalence in the community, we excluded studies in selected patient groups or those performed in hospital or allergy clinic settings and included only population-based cross-sectional and cohort studies. Reviews and case reports were



* The primary search was conducted in OVID MEDLINE and modified for EMBASE and PubMed. The search involved a combination of three search groups as either MeSH terms or keywords, each of which had to be present in order for an article to be included: 1) "nut s", "tree nuts" or an individual tree nut term; 2) "hypersensitivity" or "allergy"; and 3) "prevalence" or "epidemiology". The search was limited to English language articles. The exact search conducted in OVID MEDLINE is shown in the box below.

1. (hazelnut* or hazel nut* or cashew*or pistachio* or almond* or treenut* or tree nut* or pecan* or brazilnut* or brazil nut* or walnut*).af. 2. Nuts/ae, im, po, to 3. prevalence 4. Epidemiology 5. food hypersensitivity/ or nut hypersensitivity 6. allerg*.af. 7. (1 or 2) and (3 or 4) and (5 or 6) 8. nut hypersensitivity/ep 9. food hypersensitivity/ep and (1 or 2) 10. 7 or 8 or 9 11. limit 10 to english language

Fig. 1 Summary of the search method

excluded along with studies of which full-text articles were not available.

Identified articles were screened via title and abstract by two independent reviewers. Any discrepancies were resolved by consensus and if necessary a third reviewer consulted. Reference lists of identified studies were reviewed for additional articles. A full-text review was then undertaken for all articles identified.

Quality assessment of the studies was performed by two reviewers based on participation rate, ability of the study design to address tree nut allergy outcomes objectively and inclusion of individual tree nut information.

Analysis

Using a standardised method, relevant study details were summarised including reference details, age, sample size and response rate, prevalence estimates and 95 % confidence intervals (CI) for all reported food allergy outcomes (self-/parent report, specific IgE testing, skin prick testing, symptoms and food challenges) for overall food allergy and tree nut allergy. If not reported, prevalence estimates were calculated as the observed proportion with 95 % CI calculated on the assumption of a binomial sampling distribution.

We subclassified the prevalence estimates and 95 % CI for age, region and method of tree nut allergy diagnosis.

For this review, the approaches used to determine tree nut allergy have been grouped as follows:

1. Confirmed tree nut allergy—defined as food challenge confirmed tree nut allergy (OFC or DBPCFC) or recent history (<2 years) of *IgE-mediated reaction* with positive allergy testing (SPT or sIgE) undertaken as part of the study in the absence of a formal food challenge.
2. Probable tree nut allergy—defined as reported history (>2 years) of *IgE-mediated reaction* with allergy or self-report of doctor diagnosis (presumed to include allergy-specific history and testing).
3. Self-reported tree nut allergy—defined as parent or self-reported tree nut allergy in the absence of data on allergy testing.
4. Sensitisation only (allergy testing via SPT or sIgE, without confirmation of clinical allergy).

We performed a random effects meta-analysis and in an attempt to address the significant heterogeneity observed across the studies stratified by age, region and method of tree nut allergy diagnosis. Statistical analyses were undertaken using STATA 13 (Stata Corp, College Station, TX, USA).

Results

Study Selection and Characteristics

Figure 1 summarises the search methodology. The systematic search of the literature resulted in 333 articles after duplicates were removed. Title and abstract review identified 261 that did not meet the inclusion criteria. The remaining 72 articles and an additional ten records identified through manually searching reference lists underwent full-text review. Forty six full-text articles were excluded (26 were in selected populations, seven did not report tree nut allergy prevalence, seven did not include prevalence data and six were review articles).

Included studies are described in Table 1 (n=36).Twenty six studies were designed to measure overall food allergy prevalence and reported tree nut allergy as a study outcome, seven were studies specifically aimed at investigating tree nut allergy prevalence and three studies included tree nut allergy prevalence data as part of an investigation of peanut allergy prevalence or associated factors.

Quality assessment of the studies based on participation rate, ability of the study design to address tree nut allergy outcomes objectively and inclusion of individual tree nut information resulted in 28 studies graded as moderate and eight poor. Three of the studies were assessed as poor because they were not designed to measure tree nut allergy prevalence but reported some tree nut prevalence data, which we have included in this review. The majority (n=28) of the studies were population-based cross-sectional studies and the remaining eight were cohort studies. Six studies did not provide participation rate details, ten studies had a participation rate above 80 %, 13 between 50 and 80 % and seven less than 50 %. One study by Greenhawt et al. in American college students had a participation rate of only 3 % and reported a very high overall self-reported food allergy prevalence of 54 % and a self-reported tree nut allergy prevalence of 9.16 % (95% CI 6.8–11.9) [24]. This study has been included in the summary table, but the prevalence estimates not discussed as part of the review since the participation rate was extremely low and the study therefore not necessarily representative of the population from which it was sampled.

The random effects meta-analysis showed heterogeneity to be too great to report pooled results (I^2 >98 %, p=0.000 for all analyses).

Tree Nut Allergy Prevalence by Age and Allergy Diagnosis Method

The majority (n=24) of studies in this review were in children and adolescents, four studies included both adults and children, six studies adults only and two studies reported an overall tree nut allergy prevalence without age breakdown; in one of these studies, participants were >15 years [23] and the second <61 years of age [25].

Prevalence estimate ranges for all allergy definitions, categorised by age, are outlined in Table 2. Seven studies used the most objective assessment of oral food challenge (or convincing recent history of allergic reaction together with positive allergen-specific IgE) with an overall prevalence range of 0–1.6 %. Nine studies combined self-reported food allergy with additional objective assessment such as specific details regarding doctor diagnosis or sensitisation details (sIgE/SPT) and were classified as probable food allergy for this review. The overall probable tree nut allergy prevalence range was 0.05–4.9 %, with only one study reporting adult data. However, the majority of prevalence estimates for tree nut allergy were based on self-reported reactions (n=20 studies). The self-reported tree nut allergy prevalence range was wider for adults (0.18–8.9 %) and those studies including both adults and children (0.4–11.4 %) than those studies including only children (0–3.8 %). Overall self-reported tree nut allergy prevalence ranged from 0 to 11.4 %.

Three studies based tree nut allergy prevalence on sensitisation alone (sIgE or SPT) without any clarification of presence of clinical allergy. One reported hazelnut sensitisation by SPT in Russian children of 0.8 % (95 % CI 0.4–1.1) and Finnish children of 6.3 % (95% CI 3.6–9.8) [52]. The second study reported sensitisation based on SPT of 1.0 % in 7-year-old children in the UK [40]. The third study in adults reported sensitisation prevalence to hazelnut of 9.26 % and walnut 2.98 % (overall 12.2 % (95% CI 11.7–12.7)) [20]. This was

Table 1 Summary of the characteristics of studies in review: studies published January 1, 1996–Dec 31, 2014 (alphabetical by author)

Reference	Country	Study design	Allergy outcome	Type of allergy	*N*	Participation rate (%)	Age	Individual tree nuts described	Prevalence measure	Overall prevalence % (95 % CI) (*N*)	Study grading
Ahn et al. 2012 [17]	Korea	Cross-sectional	2. Probable (self-report of Dr diagnosis and sIgE)	Primary and secondary	7882	97	6–13 years	NA	Point and lifetime	0.05 % (0.01–0.13) (4/7882)	Moderate
Bedolla-Barajas et al. 2014 [18]	Mexico	Cross-sectional	1. Self-report	Primary and secondary	1126	NA	18–50 years	Yes	Point	0.18 % (0.02–0.64) (2/1126)	Poor
Ben-Shoshan et al. 2010 [19]	Canada	Cross-sectional	1. Self-report 2. Probable (self-report of Dr diagnosis and sIgE)	Primary and secondary	9667	34.6	All ages with breakdown	NA	Point	Children 1.1.73 (1.16–2.3) 2.0.69 (0.4–0.97) Adults: 1. 1.07 (0.84–1.30) 2. 0.35 (0.27–0.44) Overall: 1. 1.22 % (1.00–1.44) (118/9667) 2. 0.68 % (0.54–0.83)	Moderate
Burney et al. 2014 [20]	Multi (Europe)	Cross-sectional	4. Sensitisation (sIgE)	Primary and secondary	17,366	54.9	20–54 years	Yes	Point	12.2 % (11.7–12.7) (2121/17326)	Moderate
Caffarelli et al. 2011 [21]	Italy	Cross-sectional	1. Self-report	Primary and secondary	625	69	5–14 years	Yes		0.32 % (0.04–1.2) (2/625)	Moderate
DuToit et al. 2008 [22]	UK Israel	Cross-sectional	1. Self-report	Primary	4148 (UK) 4672 (Israel)	80.2 (UK) 83.2 (Israel)	4–18 years	NA	Point	UK 1.85 % (1.5–2.3) (77/4148) Israel 0.13 % (0.05–0.3) (6/4672)	Moderate
Emmett et al. 1999 [23]	UK	Cross-sectional	1. Self-report	NA	16,434	NA	All ages	NA	Point	0.40 % (0.30–0.51) (63/16,434)	Moderate
Greenhawt et. al. 2009 [24]	USA	Cross-sectional	1. Self-report	NA	571	3.5	>18 years	NA	Point	9.16 % (6.8–11.9) (47/571)	Poor
Kanny et al. 2002 [25]	France	Cross-sectional	1. Self-report	Primary and secondary	16,174	52	All ages <60	NA	Point	3 % (2.7–3.20)	Moderate
Kaya et al. 2013 [26]	Turkey	Cross-sectional	1. Self-report 3. Confirmed (DBPCFC)	Primary	10,096	89.9	11–15 years	Yes	Lifetime	1.1.2 % (0.1–1.4) (121/10,096) 3.0.05 % (0.02–0.1) (6/100,096)	Moderate
Kljakovic et al. 2009 [27]	Australia	Cross-sectional	1. Self-report	NA	3851	85	4–5 years	No	Lifetime	1.79 % (1.4–2.3) (69/3851)	Poor
Kristjansson et al. 1999 [28]	Sweden Iceland	Cross-sectional	1. Self-report 3. Confirmed (OFC)	NA	324 (Iceland) 328 (Sweden)	79 (Iceland) 90 (Sweden)	18 months	No	Point	1. Sweden 0.3 % (0.0–1.6) (1/328) Iceland 0 % 3.0 % for Iceland and Sweden	Moderate

Table 1 (continued)

Reference	Country	Study design	Allergy outcome	Type of allergy	*N*	Participation rate (%)	Age	Individual tree nuts described	Prevalence measure	Overall prevalence % (95 % CI) (*N*)	Study grading
Leung et al. 2009 [29]	Hong Kong	Cross-sectional	1. Self-report 2. Probable (self-report of Dr diagnosis)	Primary and secondary	3677	83.6 %	2–7 years	NA	NA	1. 0.41 (0.2–0.7) (15/3677) 2. 0.3 % (0.2–0.5) (11/3677)	Moderate
Marklund et al. 2004 [30]	Sweden	Cross-sectional	1. Self-report	Primary and secondary	1451	97	13–21 years	NA	Point	11.37 % (9.5–12.8) (165/1451)	Moderate
Mustafayev et al. 2012 [31]	Turkey	Cross-sectional	1. Self-report 2. Probable (detailed history and SPT) 3. Confirmed (OFC)	Primary	6963	NA	10–11 years	Yes	Point	1.3.5 % (3.1,3.9) (223/6963) 2.4.9 % (4.4,5.4) (341/6963) 3.0.05 % (0.03,0.15) (4/6963)	Moderate
Nicolaou et al. 2010 [32]	UK	Cohort	1. Self-report	Primary	1029	94.9	8 years	NA	Lifetime	1.0 % (0.4,1.8) (10/1029)	Poor
Orhan et al. 2009 [33]	Turkey	Cross-sectional	1. Self-report 2. Probable (SPT) 3. Confirmed (OFC)	Primary	2739	78.2	6–9 years	Almond and walnut	Point	1.0.4 % (0.2,0.7) (11/2739) 2.0.14 % (0.03,0.4) (4/2739) 3.0 % (0,0.1) (0/2739)	Moderate
Ostblom et al. 2008 [34]	Sweden	Cohort	1. Self-report	Primary	2563	69	4 years	NA	Point	3.8 % (3.1,4.6) (98/2563)	Moderate
Osterballe et al. 2009 [35]	Denmark	Cohort	1. Self-report	Specified primary and secondary	843	77	22 years	Yes	Point	1. Primary 0 % Secondary 8.9 % (7.0,11.02) (85/843)	Moderate
Penard-Morand et al. 2005 [36]	France	Cross-sectional	1. Self-report	Primary and secondary	7781	81	9–11 years	NA	Lifetime	0.2 % (0.1,0.3) (10/6672)	Moderate
Pereira et al. 2005 [37]	UK	Cohort	1. Self-report	Primary and secondary	1532	48.7	11 and 15 years	NA	Point	1.6 % (1.1,2.4) (26/1532)	Poor
Pyrhonen et al. 2005 [38]	Finland	Cross-sectional	1. Self-report 2. Probable (self-report of Dr diagnosis)	Primary and secondary	3308	69	1–4 years	NA	Lifetime	1.1.5 % (1.1,1.9) (49/3308) 2.0.2 % (0.08,0.4) (7/3308)	Moderate
Rance et al. 2005 [39]	France	Cross-sectional	1. Self-report	Primary and secondary	2716	77.6	2–14 years	Yes	Point	0.7 % (0.4,1.1) (19/2716)	Moderate
Roberts et al. 2005 [40]	UK	Cohort	4. Sensitisation (SPT)	Sensitisation only	5848	42	7 years	Yes	Point	1.04 % (0.8,1.3) (61/5848)	Poor
Roehr et al. 2004 [41]	Germany	Cross-sectional	1. Self-report 2. Probable (SPT) 3. Confirmed (OFC)	Primary and secondary	739	31.5	0–17 years	Yes	Point	1. NA 2. 2.7 % (1.6,4.1) (20/739) 3. 1.4 % (0.7,2.5) (10/739)	Moderate

Table 1 (continued)

Reference	Country	Study design	Allergy outcome	Type of allergy	*N*	Participation rate (%)	Age	Individual tree nuts described	Prevalence measure	Overall prevalence % (95 % CI) (*N*)	Study grading
Schafer et al. 2001 [42]	Germany	Cross-sectional	1. Self-report SPT for hazelnut only	Primary and secondary	1537	60.7	25–74 years	NA	Point	1.8.5 % (7.1,9.9) (130/1537)	Moderate
Shek et al. 2010 [43]	Singapore Philippines	Cross-sectional	1. Self-report 2. Probable (self-report of Dr diagnosis)	Primary	25,692	74.2	4–6 years and 14–16 years	NA	Point	1.1.85 % (1.6,2.1) (200/10775) 2.0.28 % (0.2,0.4) (31/10,775)	Moderate
Sicherer et al. 1999 [44•]	USA	Cross-sectional	1. Self-report	Primary and secondary	4374	62	All ages with breakdown	Yes	Point	Children (<18 years) 0.2 % (0.05,0.4) (5/2998) Adults (>18 yrs) 0.7 % (0.5,0.9) (59/8049) Overall 0.5 % (0.0,0.6) (64/12032)	Moderate
Sicherer et al. 2003 [45•]	USA	Cross-sectional	1. Self-report	Primary and secondary	13,493	52	All ages with breakdown	Yes	Point	Children (<18 years) 0.2 % (0.1,0.4) (7/3127) Adults (>18 years) 0.1 % (0.4,0.6) (50/9881) Overall 0.4 % (0.3,0.5) (57/13,493)	Moderate
Sicherer et al. 2010 [46•]	USA	Cross-sectional	1. Self-report	Primary and secondary	5300	42	All ages with breakdown	Yes	Point	Children (<18 years) 1.1 % (0.05,0.4) (31/2902) Adults (>18 years) 0.5 % (0.4,0.6) (53/9845) Overall 0.6 % (0.5,0.8) (84/12,658)	Moderate
Tariq et al. 1996 [47]	UK	Cohort	1. Self-report Some participants had SPT	Primary	1218	NA	4 years	NA	Point	0.1 % (0.02,0.6) (2/1218)	Poor
Taylor-Black et al. 2014 [48]	USA	Cross-sectional	1. Self-report	Primary	368	43	4–12 years	NA	Point	1.82 % (1.06,2.9) (17/932)	Poor
Venter et al. 2006 [49]	UK	Cohort	1. Self-report 3. Confirmed (OFC)	Primary	798	55.4	6 years	Yes	Point	1. 1.37 % (0.8,2.5) (11/798) 3. 0.25 % (0.03,0.9) (2/798)	Moderate

Table 1 (continued)

Reference	Country	Study design	Allergy outcome	Type of allergy	*N*	Participation rate (%)	Age	Individual tree nuts described	Prevalence measure	Overall prevalence % (95 % CI) (*N*)	Study grading
Venter et al 2008 [50]	UK	Cohort	1. Self-report 3. Confirmed (OFC)	Primary	891	91.9	3 years	Yes	Point	3.0.93 % (0.34,2.0) (6/642)	Moderate
Vierk et al. 2007 [51]	USA	Cross-sectional	1. Self-report 2. Probable (self-report of Dr diagnosis)	Primary	4482	NA	>18 years	NA	Point	1.0.65 % (0.43,0.9) (29/4477) 2.0.5 % (0.06,0.32) (7/4477)	Moderate
Von Hertzen et al. 2006 [52]	Finland Russia	Cross-sectional	4. Sensitisation (SPT)	Primary and secondary	Finland 367 Russia 446	NA	7–16 years	Hazelnut only	Point	Finland 6.3 % (3.6,9.8) (17/271) Russia 0.8 % (0.2,2.4) (3/356)	Moderate

the highest reported prevalence estimate of all four methods of tree nut allergy definition.

Tree Nut Allergy Prevalence by Region

Prevalence estimate ranges for each method of allergy definition are summarised by region in Table 3. Regional variation in self-reported tree nut allergy prevalence is illustrated in Fig. 2. Most studies were from Europe (n=18), the UK (n=8), or the USA (n=5). There were three studies from Asia and one each from Canada, Central America and Australia. Stratifying by region highlighted a markedly higher prevalence of tree nut allergy in some European countries with a range of 0.04–11.4 %. OAS appeared to contribute to higher tree nut allergy prevalence in some European countries since all three of the studies reporting tree nut allergy prevalence over 8 % were self-reported, all in adolescents and adults, and all from Europe. Two of these studies directly reported that all tree nut allergy found in their study was due to OAS [35, 42] and the third study did not specify the type of allergic reaction to tree nuts, but overall 33 % of all allergy, to any food, was reportedly due to OAS [30]. All other regions, regardless of allergy definition, reported tree nut allergy prevalence less than 2 %.

Individual Tree Nut Allergy Prevalence

Table 4 summarises the percentage of tree nut allergic participants allergic to each individual tree nut by region. Fourteen studies provided details of individual tree nut prevalence. The prevalence of individual tree nut allergies varied by region. Hazelnut was the most common tree nut allergy reported in six of the seven studies from Europe accounting for 17–100 % of all tree nut allergies. The two studies from the USA reported walnut and cashew as the most common tree nut allergies ranging from 20 to 30 % and 15–30 %, respectively. Brazil nut allergy was reported commonly in the UK ranging from 24 to 33 %. The one study from Mexico reported low overall tree nut allergy of 0.18 % (2/1126) with both participants allergic to walnut. None of the studies reported on the prevalence of multiple tree nut allergies.

Tree Nut Allergy Prevalence Over Time

There is limited evidence to determine if the population prevalence of tree nut allergy is increasing. Three studies in the USA utilised random-digit telephone surveys in 1997, 2002 and 2008 [44•, 45•, 46•]. Study design was consistent across each sampling period and included a large number of participants (n=4374; 13,493 and 5300, respectively). No significant increase in adult self-reported tree nut allergy prevalence was found over the three time points. However, the prevalence of self-reported tree nut allergy in children younger than 18 years had increased

Table 2 Summary of the range of prevalence estimates of tree nut allergy in the reviewed studies according to allergy assessment method and age

Allergy definition and age	Number of studies	Range of prevalence estimates (%)	References
Self-reported			
Children 0–18 years	22	0–3.8	[21, 22, 26–29, 31–34, 36–39, 43, 44•, 45•, 46•, 47–49, 53]
Adult	8	0.18–8.9	
All ages	3	0.4–11.4	[18, 35, 42, 44•, 45•, 46•, 51, 53]
Overall		0–11.4	[23, 25, 30]
Probable			
Children 0–18 years	9	0.05–4.9	[17, 19, 29, 31, 33, 38, 41, 43, 51]
Adult	2	0.35–0.5	[19, 51]
All ages	0	NA	
Overall		0.05–4.9	
Confirmed			
Children 0–18 years	7	0–1.4	[26, 28, 31, 33, 41, 49, 50]
Adult	0	NA	
All ages	0	NA	
Overall		0–1.4	
Sensitisation			
Children 0–18 years	2	0.8–6.3	[40, 52]
Adult	1	12.2	[20]
All ages	0	NA	
Overall		0.8–12.2	

Some studies are included in more than one category as they reported prevalence estimates obtained using more than one allergy assessment method.

significantly (0.2 % in 1997, 0.5 % in 2002 and 1.1 % in 2008). Proportionally, the increase was greater than that observed for peanut over the same time periods (0.4 % in 1997, 0.8 % in 2002 and 1.4 % in 2008).

Table 3 Summary of the range of reported prevalence estimates for tree nut allergy according to allergy assessment method and region

Region		Self-report Range % (number of studies)	Probable Range % (number of studies)	Confirmed Range % (number of studies)	Sensitisation Range % (number of studies)
Asia	Children	0.3–1.85 (3)	0.05–0.3 (3)	NA	NA
	Adults	NA	NA	NA	NA
	Overall	0.3–1.85	0.05–0.3	NA	NA
Europe	Children	0.04–3.1 (10)	0.2–4.9 (4)	0–1.4 (6)	0.8 (1)
	Adults	8.5–8.9 (2)	1.6 (1)	NA	12.2 (1)
	All ages	3.0–11.7	NA	NA	NA
	Overall	0.04–11.7	0.2–4.9	0–1.4	0.8–12.2
UK	Children	0.1–1.85 (5)	NA	0.25–0.93 (2)	NA
	Adults	NA	NA	NA	NA
	Overall	0.1–1.85		0.25–0.93	
USA	Children	0.2–1.82 (4)	NA	NA	NA
	Adults	0.5–0.7 (2)	NA	NA	NA
	Overall	0.2–1.82			
Australia	Children	1.79 (1)	NA	NA	NA
	Adults	NA	NA	NA	NA
	Overall				
Canada	Children	1.73 (1)	1.59 (1)	0.69 (1)	NA
	Adults	1.07 (1)	1.0 (1)	0.35 (1)	NA
	Overall	1.07–1.73	1.0–1.59	0.35–0.69	
Central America	Children	NA	NA	NA	NA
	Adults	0.02 (1)	NA	NA	NA
	Overall				

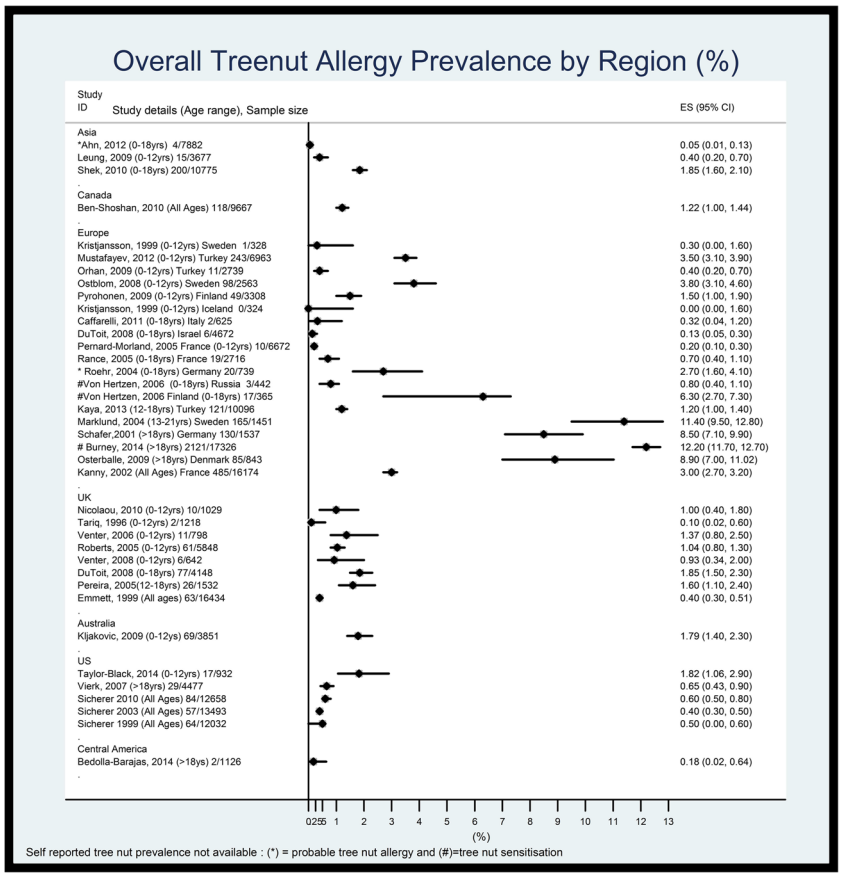


Fig. 2 Overall tree nut allergy prevalence by region (%)

Table 4 Percentage of tree nut allergics reporting reactions to the individual tree nuts by region

Region, study details (country)	% of tree nut allergics reporting reactions to the individual tree nuts (number with specific tree nut allergy/total number with any tree nut allergy)
Europe	
Burney et al. 2014 [20] (multi-country)	Hazelnut 76 % (1605/2121), walnut 24 % (517/2121)
Caffarelli et al. 2011 [21] (Italy)	Hazelnut 100 % (2/2)
Mustafayev et al. 2012 [31] (Turkey)	Hazelnut 42 % (104/243), walnut 34 % (83/243), pistachio 22 % (55/243)
Kaya et al. 2013 [26] (Turkey)	Walnut 66 % (4/6), hazelnut 17 % (1/6), pistachio 17 % (1/6)
Osterballe et al. 2009 [35] (Denmark)	Hazelnut 75 % (56/75), Brazil nut 31 % (23/75), walnut 5 % (4/75), almond 3 % 2/75)
Rance et al. 2005 [39] (France)	Hazelnut 53 % (10/19), walnut 32 % (6/19), almond 10 % (2/19), cashew 5 % (1/19)
Roehr et al. 2004 [41] (Germany)	Hazelnut 100 % (10/10)
USA	
Sicherer et al. 1999 [44•]	Walnut 37 % (24/65), cashew 12 % (5/65), Brazil nut 12 % (8/65), almond 11 % (7/65), pecan 11 % (7/65), hazelnut 4.6 % (3/65), macadamia 3 % (2/65), unspecified 9 % (6/65)
Sicherer et al. 2010 [46•]	Walnut 48 % (41/84), cashew 34 % (29/84), pecan 30 % (26/84), almond 29 % (25/84), pistachio 22 % (19/84), Brazil nut 22 % (19/84), hazelnut 20 % (17/84), macadamia 20 % (17/84), pine nut 13 % (11/84)
UK	
Venter et al. 2008 [50]	Brazil nut 33 % (2/6), almond 33 % (2/6), hazelnut 17 % (1/6), cashew 17 % (1/6)
Venter et al. 2006 [49]	Almond 33 % (1/3), Brazil nut 33 % (1/3), hazelnut 33 % (1/3)
Roberts et al. 2005 [40]	Walnut 24 % (10/41), Brazil nut 24 % (10/41), almond 22 % (9/41), cashew 15 % (10/41), hazelnut 7 % (3/41), pecan 7 % (3/41)
Tariq et al. 1996 [47]	Hazelnut 50 % (1/2), cashew 50 % (1/2)
Mexico	
Bedolla-Barajas et al. 2014 [18]	Walnut 100 % (2/2)

Discussion

This review has confirmed that the majority of tree nut allergy prevalence studies continue to be undertaken in Europe, where there is a high prevalence of OAS, with most studies relying on self-reported prevalence, limited to children and adolescents. Using the most robust measure of tree nut prevalence (challenge confirmed or history of reaction with IgE antibodies), we estimate the overall prevalence to be <2 % in countries where OAS is not reported. Secondary tree nut allergy (OAS) estimates for older age groups including adolescents and adults is as high as 10 %, particularly in Europe. Few studies reported the population prevalence of individual tree nut allergies. However, how prevalent a particular tree nut allergy is differs significantly by region with hazelnut the most common tree nut allergy in Europe, walnut and cashew in the USA and Brazil nut, almond and walnut most commonly reported in the UK. There is limited evidence to determine if the population prevalence of tree nut allergy is increasing.

This is the first systematic review of the literature exploring tree nut allergy prevalence exclusively across the age groups and different regions of the world, utilising robust systematic review methodology, closely following PRISMA guidelines. A further strength of this review is we categorised prevalence by robustness of the study methodology employed to define tree nut allergy. We identified three studies with self-reported tree nut allergy greater than 8 %, all from Europe demonstrating that studies which do not differentiate primary and secondary tree nut allergy prevalence rates are likely to inflate prevalence estimates.

Precise estimates of true tree nut allergy were limited by the small number of studies reporting challenge confirmed tree nut allergy prevalence—the gold standard for diagnosis. As for other epidemiological studies of food allergy prevalence [8•, 9], we also found higher prevalence estimates for self-report and sensitisation. Self-report is known to overestimate the true prevalence of food allergy [54] and asymptomatic sensitisation to foods is relatively common [9]; therefore, objective measures are critical. We were also unable to accurately determine whether tree nut allergy is on the rise as only one series of estimates was available. Finally, estimates of the prevalence of individual tree nut allergies could not be reliably estimated due to the paucity of data reported for individual nuts, although it is clear that there is significant regional variation in prevalence estimates [55].

We found overall tree nut allergy prevalence mirrors the global pattern of overall food allergy with countries with low prevalence of food allergy also reporting low levels of tree nut allergy. Large population-based epidemiological studies such as the ISAAC and EuroPrevall studies have demonstrated considerable regional variability of common food allergens and sensitisation patterns, but the reasons for this remain largely unexplored. It has been hypothesised that variation in dietary patterns at the population level might lead to variations in sensitisation status and hence risk of subsequent food allergy. Du Toit et al. hypothesised that variations in peanut allergy prevalence between genetically similar populations in the UK and Israel might be due to differences in infantile peanut consumption patterns [22], whilst others have argued that boiled versus roasted peanut dietary intakes may at least partly explain the difference in allergy patterns across different regions [56, 57].

Our review found a higher self-reported tree nut allergy range (0–11.4 %) than both previous published systematic reviews. Nwaru et al. performed a meta-analysis of seven studies and reported a pooled self-reported point prevalence of 1.8 % (95% CI 1.63–1.99), although there was significant heterogeneity across the studies (I^2=99.4 %, p=0.00). Zuidmeer et al. included studies from a wider range of countries from 1990 to 2006 and reported a self-reported tree nut allergy prevalence range of 0–7.3 % based on seven studies [12•]. Prevalence varied based on type of tree nut allergy, method of tree nut allergy diagnosis, age and region. Similarly to Zudimeer et al., considering the large heterogeneity between the studies, we have not presented a pooled prevalence estimate since this would mask the differences between populations.

Nwaru et al. reported confirmed tree nut allergy pooled point prevalence of 0.45 % (I^2=0.00 %, p=0.88) while Zuidmeer et al. reported a range of 0.1–4.3, based on only three studies. We found the prevalence of tree nut sensitisation to be the highest of the four methods of allergy definition used (1.0–12.2 %). Comparison to sensitisation prevalence estimates in previous reviews is difficult as we reported sensitisation prevalence estimates based on population sensitisation. Previous reviews both reported studies where SPT or sIgE was performed only on participants that had previously self-reported tree nut allergy. Neither of these reviews differentiated between primary and secondary tree nut allergy prevalence, or reported on individual tree nut allergy prevalence nor the nature or prevalence of multiple tree nut allergies.

In conclusion, this systematic review has highlighted that there is considerable heterogeneity in tree nut allergy prevalence from studies to date and pooling individual study estimates risks masking the real differences between populations. Data is limited to largely European, US and UK studies using self-reported prevalence in children and adolescents. There is a need for further studies to determine tree nut allergy by gold standard methodologies such as food challenge, and differentiate between primary and secondary tree nut allergy. Further detailed information on individual tree nut prevalences will help inform our understanding of regional variation and repeated estimates over time will enable us to understand whether time trends in tree nut allergy mirror the general rise in IgE-mediated food allergy reported in developed countries.

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Compliance with Ethics Guidelines

Conflict of Interest Drs McWilliam, Koplin, Lodge, Tang, Dharmage and Allen declare no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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5.ADVERSE REACTIONS TO TREE NUTS

5.1 Introduction

This chapter is presented as a manuscript that was published in The Journal of Clinical Immunology and Allergy (2018; 141:982-90.), the highest ranked allergy journal internationally with an impact factor of 12.485.

This is one of the first studies to investigate and characterise adverse food reactions including anaphylaxis and associated risk factors in the adolescent period, an age group identified at high risk for adverse food reactions and anaphylaxis but poorly studied in food allergy research.

This manuscript addresses the following research questions:

Do food allergic sufferers have more reactions or severe reactions when peanut or tree nut allergic versus other food allergies?

a) What is the prevalence of self-reported adverse reactions for peanut, tree nut and other foods in 10 to 14 year old allergic children in the SchoolNuts study?
b) What is the prevalence of self-reported anaphylaxis for peanut, tree nut and other foods in 10 to 14 year old allergic children in the SchoolNuts study?
c) What factors are associated with adverse food allergy reactions and anaphylaxis in 10 to 14 year old allergic children in the SchoolNuts study?

This research was presented at the European Academy of Allergy and Clinical Immunology (EAACI) Congress in Vienna, Austria in June 2016 as an oral presentation. It was awarded best oral presentation in the anaphylaxis section of the meeting. EAACI is Europe's largest medical association in the fields of allergy and clinical immunology and the congress is their annual meeting with around 8000 delegates attending each year.

5.2 The Manuscript

Food allergy and gastrointestinal disease

Self-reported adverse food reactions and anaphylaxis in the SchoolNuts study: A population-based study of adolescents

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Background: Adolescents are at the highest risk of death from anaphylaxis, yet few population-based studies have described the frequencies and risk factors for allergic reactions caused by accidental allergen ingestion in this group.

Methods: We describe the prevalence, frequency, and associated risk factors for recent adverse food reactions in 10- to 14-year-olds in Melbourne, Australia, recruited from a stratified, random, population-based sample of schools (SchoolNuts, n = 9663; 48% response rate). Self-reported food allergy and adverse reaction details, including anaphylaxis, were identified by using a student questionnaire over the past year.

Results: Of 547 students with possible IgE-mediated food allergy, 243 (44.4%; 95% CI, 40.3% to 48.7%) reported a reaction to a food. Fifty-three (9.7%; 95% CI, 7.2% to 12.2%) students reported 93 anaphylaxis episodes. Peanut and tree nuts were the most common food triggers. Among students with current IgE-mediated food allergy, those with resolved or current asthma (adjusted odds ratio [aOR], 1.9 [95% CI, 1.1-1.3] and 1.7 [95% CI, 1.1-2.6]) and those with more than 2 food allergies (aOR, 1.9 [95% CI, 1.1-3.1]) were at greatest risk of any adverse food reaction, and those with nut allergy were most at risk of severe reactions (aOR, 2.9 [95% CI, 1.1-4.4]). Resolved or current asthma was not associated with increased risk of severe reactions (aOR, 0.8 [95% CI, 0.3-2.2] and 1.6 [95% CI, 0.7-3.7]).

Conclusions: Adolescents with food allergy are frequently exposed to food allergens. Those with asthma and more than 2 food allergies were at the greatest risk for adverse food reactions. Those with nut allergies were most at risk of severe reactions. (J Allergy Clin Immunol 2018;141:982-90.)

Key words: *Food allergy, anaphylaxis, adolescents, schools, peanut allergy, asthma*

Food allergy affects up to 10% of children and 2% to 3% of adults and appears to be increasing in prevalence.[1-4] This increase in food allergy prevalence has coincided with increased reports of food-induced anaphylaxis. The greatest increase in anaphylaxis has been reported in the 0- to 5-year age group[5]; however, more recent data suggest that the increase in food-induced anaphylaxis is now occurring more rapidly in the periadolescent age period (5-14 years).[6,7] Adolescents also constitute the highest proportion of deaths from anaphylaxis.[8-11]

Studies to date reporting the frequency of adverse food reactions and anaphylaxis have been limited to selected food allergens only,[12-19] occurred in younger children,[20] or been based on allergy clinic or health care facility presentation.[21] Few population studies have described the frequency of adverse food reactions, including anaphylaxis, among adolescents.

Additionally, predicting those adolescents with food allergy who might be at greatest risk of food reactions is also difficult.[22] Reported risk factors for anaphylaxis-related fatalities have included delayed administration or failure to administer

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Abbreviations used
aOR: Adjusted odds ratio
OR: Odds ratio

adrenalin,[23] having peanut or tree nut allergy (70% to 90% of anaphylaxis-related deaths are due to these foods),[8-11,24] and having coexisting or poorly controlled asthma.[23] However, these factors have not been explored formally in a population setting or in the high-risk adolescent age group. Understanding the predictors for adverse food reactions, including anaphylaxis, for the population of adolescents with food allergy underpins the development of age-appropriate prevention and management strategies.

The aim of this study was to determine the frequency and associated risk factors for adverse food reactions reported in the preceding 12 months among those with IgE-mediated food allergy in a large population-based cohort of 10- to 14-year-old students.

METHODS

The SchoolNuts study is a cross-sectional population-based study that aimed to determine the population prevalence of challenge-proved food allergy and to assess risk factors for adverse reactions to foods in early adolescence. The early adolescent period (10-14 years) was selected to allow assessment of children across the transition from primary (10-11 years) to secondary school (12-14 years), where greater autonomy and independence occurs, which is also consistent with the onset of puberty. The study had 2 stages: stage 1, parent and student questionnaires; stage 2, clinic-based assessments. Questionnaires were used to determine the likelihood of IgE-mediated food allergy, after which the students and their parents were invited to attend a hospital clinic visit for allergy testing and food challenge. This study reports the data from stage 1 student questionnaires.

Participant selection and recruitment

Stratified cluster sampling of primary and secondary school students in the greater metropolitan Melbourne area (population of 3.9 million in 2011)[25] was used to recruit a sample of 10- to 14-year-old adolescents between July 2011 and December 2014. Seventy percent of the population of the state of Victoria reside in Melbourne.[25] Schools were randomly selected to reflect the variation in socioeconomic status throughout the various school districts and included each of the government, Catholic, and independent school sectors in the Australian education system. Schools were eligible for inclusion if they were less than 80 km from the central business district and had more than 20 students per year level. A list of schools was obtained from the 2010 Melbourne Street Directory stratified by primary (grades 5 and 6) versus secondary (years 7 and 8) schools and subdivided into government, Catholic, and independent schools. Each school within those groups was then assigned a number, and a random number generator was used to select schools. These 229 schools (representing 20,965 students) were approached to participate by letter of invitation, 117 (51.1%) of which participated in the study. At each participating school, all students in years 5, 6, 7, and 8 were then invited to take part.

Parental consent was required for students to complete the student questionnaire. Consented students completed questionnaires as part of a school visit by the research team (n = 9,663). Families were invited to complete a parent questionnaire sent through the school. Fifty-seven percent of student questionnaires had a corresponding parent questionnaire completed (n = 5,507).

Questionnaire details and definitions

The student questionnaire included questions regarding the child's history of food allergy and asthma and knowledge and attitudes toward food allergy. Student-reported resolved asthma was defined as an affirmative response to the following question: "Have you ever had asthma?" Current asthma was defined as an affirmative response to the following question: "Do you still have asthma?"

Students reporting current food allergy, current asthma, or both were asked to complete additional more detailed sections of the questionnaire regarding their asthma, food allergy, or both. Those with current food allergy were asked to specify foods to which they were allergic: peanut, other nuts (nut type specified), hen's egg, cow's milk, sesame, fish, shellfish, soy, and other food (specified). Those students who did not complete further food allergy questions and those who reported a food allergy that was unlikely to be IgE-mediated (celiac disease, lactose intolerance, or reactions to additives) were excluded from the current analyses. The remaining students were classified as "possibly IgE-mediated food allergic."

Students were asked whether they had experienced an adverse food reaction in the past 12 months (see Fig E1 in this article's Online Repository at www.jacionline.org for details of the questionnaire). Details of each food reaction were sought, including the food trigger, self-report of anaphylaxis, use of adrenaline autoinjector, locale of the reaction (home, school, restaurant, and friend's home), symptoms (skin rash, facial swelling, vomiting, diarrhea, and breathing difficulties), and time frame of each reaction (<1 hour from food ingestion and reaction, 1-4 hours, >4 hours, and unknown). The definition of anaphylaxis was based on the Australasian Society of Clinical Immunology and Allergy definition of anaphylaxis, which categorizes any reaction involving acute onset of respiratory compromise as anaphylaxis (Australasian Society of Clinical Immunology and Allergy definition was any acute onset illness with typical skin features [urticarial rash or erythema/flushing, and/or angioedema] PLUS involvement of respiratory and/or cardiovascular and/or persistent severe gastrointestinal symptoms OR any acute onset of hypotension or bronchospasm or upper airway obstruction where anaphylaxis is considered possible, even if typical skin features are not present).[26] Each reaction was categorized into one of 5 groups according to time of onset of reaction and symptoms (see Fig 1 for details): (1) confirmed anaphylaxis; (2) unrecognized anaphylaxis; (3) likely IgE mediated; (4) unlikely IgE mediated; and (5) unable to be classified. V.L.M. initially reviewed and classified all cases. Dr Allen reviewed all categorizations, and any discrepancies were resolved by Dr Tang.

Students reporting multiple reactions were assigned based on the most severe reaction reported. Fig 1 shows categorization of students based on participation and classification of each reaction.

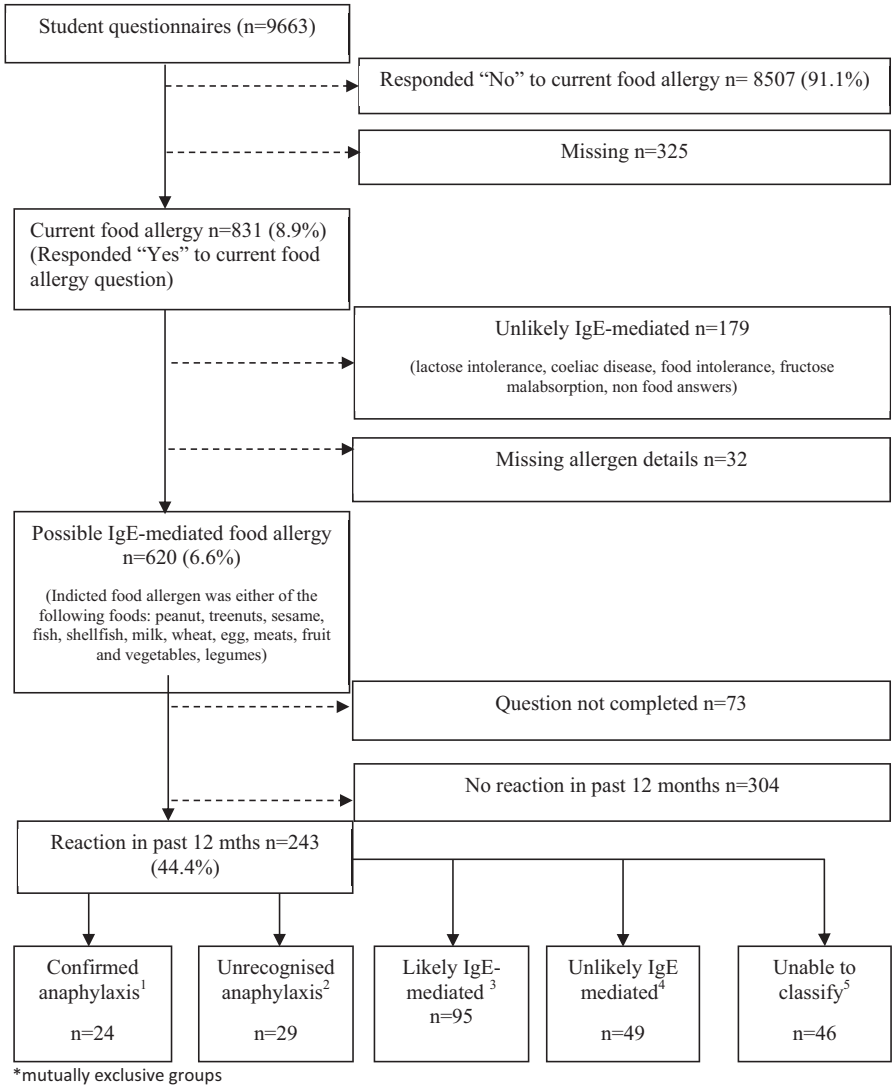
The parent questionnaire replicated questions regarding asthma and food allergy status included in the student questionnaire; however, details of food allergy reactions in the past 12 months were not included in the parent questionnaires.

Statistical methods

Self-reported possible food allergy prevalence was expressed as the observed proportion with 95% CIs. Kappa analysis was performed to measure the agreement between dyads of student and parent responses for self-reported food allergy. The prevalence of adverse reactions was calculated as the number of students reporting a reaction in the past 12 months divided by those with probable IgE-mediated food allergy.

We used logistic regression models to first identify risk factors for having adverse food reactions among those with possible IgE-mediated food allergy. Second, among those with adverse food reactions, we assessed risk factors for severe compared with mild reactions.

Multivariable logistic regression was used to examine the association between demographic factors (sex, school type, school sector, and student's country of birth) and clinical factors of interest (resolved and current asthma, number of food allergies, and type of food allergies) and the risk of adverse food reactions expressed as odds ratios (ORs) and 95% CIs. A separate regression model was conducted for each clinical factor, with the demographic factors included as *a priori* confounders and other clinical factors retained only as confounding variables if they altered the OR by greater than 10%. STATA software was used for all statistical analyses (release 14.0; StataCorp, College Station, Tex).



*mutually exclusive groups

1. Confirmed anaphylaxis – student stated the reaction was anaphylaxis and symptoms included breathing difficulties in less than 1 hour.,
2. Unrecognised anaphylaxis – Student reported reaction was not anaphylaxis or they did not know or didn't answer but symptoms included breathing difficulties and occurred in less than one hour.
3. Likely IgE mediated – student stated the reaction was not anaphylaxis, symptoms (skin rash, facial swelling, vomiting, diarrhoea, breathing difficulties occurred within 1-4 hours and breathing difficulties were not reported as a symptom.
4. Unlikely IgE mediated – symptoms were >4 hours and/or included diarrhoea as the only symptom.
5. Unable to be classified – insufficient reaction details were completed to be able to classify the reaction type.

FIG 1. Overview of student participation in the SchoolNuts study.

Ethical approval was obtained from the Royal Children's Hospital Human Research and Ethics Committee, the Department of Education and Early Childhood Development, and the Catholic Education Office.

RESULTS

Study population

Table I outlines the demographic characteristics of the study population. The mean age was 12 ± 1.2 years, with balanced participation in terms of sex (50.5% males) and school level (51.7% attended primary school). Nearly half the students (47.3%) attended government schools, which matches the school sector breakdown in Victoria.[27] A total of 831 students reported current food allergy (8.9%; 95% CI, 8.3% to 9.5%), with 620 (6.6%; 95% CI, 5.6% to 6.9%) classified as having a possible IgE-mediated food allergy (Fig 1). Kappa analysis showed a strong correlation between student and parent responses for self-reported food allergy (0.75 [95.8%]). Current asthma was reported by 177 (32.4%; 95% CI, 28.5% to 36.3%) of those with possible IgE-mediated food allergy (n = 547), 33.7% (95% CI, 27.8% to 39.6%) of those reporting any reaction (n = 243; Table II), and 45.2% (95% CI, 40.4% to 48.7%) of those reporting reactions that met the criteria for anaphylaxis in the past 12 months (n = 53; Table III). Peanut (n = 294) and tree nut

TABLE I. Demographic description of the SchoolNuts study population (n = 9663)

Demographic description	No.	Percentage
Sex		
Male	4878	50.5
Female	4785	49.5
Age		
Mean (SD [y])	11.9 (1.2)	—
Median (range [y])	12.0 (9-15)	—
Student's country of birth*		
Australia	8233	85.7
Other	1380	14.3
School level		
Primary	4994	51.7
Secondary	4669	48.3
School type (ICSEA), mean (SD)†		
Catholic (1082 [77.2])	2871	29.7
Government (1001 [49.1])	4619	47.8
Independent (1148.6 [42.5])	2173	22.5

*Numbers do not add up to total because of missing data.
†The Index of Community Socio-educational Advantage *(ICSEA)* scale for each school is generated from student-level parent occupation and education, location, and percentage indigenous student enrollment, as developed by the Australian Curriculum, Assessment and Reporting Authority.

(n = 249) were the most commonly reported food allergies at 3.0% (95% CI, 2.8% to 3.5%) and 2.6% (95% CI, 2.3% to 3.0%), respectively.

Details of food allergy reactions in the past 12 months among those with possible IgE-mediated food allergy

Table IV details all episodes of food-induced allergic reactions reported in the 12 months before the study. Among those students with possible IgE-mediated food allergy (n = 547), a total of 372 allergic reactions were reported by 243 (44.4%; 95% CI, 40.3% to 48.7%) students. More than 1 reaction was reported by 34% of students (n = 73). A total of 53 students reported 93 reactions that met the criteria for anaphylaxis (confirmed anaphylaxis [n = 44] and unrecognized anaphylaxis [n = 49]) over the 12-month period, resulting in a prevalence of 9.7% (95% CI, 7.2% to 12.2%).

Peanut and tree nut were the reported trigger foods for 43.8% (106/242) of all IgE-mediated reactions and 57% (53/93) of all severe reactions (confirmed and unrecognized anaphylaxis). Cashew and walnut were the most common tree nuts reported. Home was the location for 37 (39.8%) of the 93 episodes of all types of anaphylaxis (confirmed and unrecognized anaphylaxis), whereas restaurants were the location for 17% (n = 16) and schools for 8.6% (n = 8). Of the 44 episodes of confirmed anaphylaxis, adrenaline autoinjectors were used for 19 (43.2%) episodes.

Risk factors for reporting an adverse food reaction in the past 12 months among those with a possible IgE-mediated food allergy

Table II presents the risk factor analysis for having an adverse reaction to a food in the last 12 months among those with possible IgE-mediated food allergy (n = 547). Adjusted multivariable analysis showed that female subjects (adjusted odds ratio [aOR] 1.9; 95% CI, 1.3-2.5), those with either a history of asthma (aOR, 1.9; 95% CI, 1.1-3.2) or current asthma (aOR, 1.7; 95% CI, 1.1-2.6), and those with more than 2 food allergies (vs single food allergy: aOR, 1.9; 95% CI, 1.1-3.8) were more likely to report an adverse food reaction in the past 12 months. Those with nut allergy were less likely to report a food reaction in the past 12 months than those without nut allergy (aOR, 0.7; 95% CI, 0.5-0.8).

Risk factors for a food allergy reaction in the past 12 months that met the criteria for anaphylaxis among those with IgE-mediated food allergy reporting a reaction

We compared those who reported a nonsevere IgE-mediated reaction in the past 12 months (n = 95) with those who reported a reaction consistent with anaphylaxis (confirmed or unrecognized anaphylaxis, n = 53). There was no association between risk of anaphylaxis and demographic factors, current or resolved asthma, and number of food allergies. However, those with nut allergy were almost 3 times more likely to report anaphylaxis in the past 12 months than those without nut allergy (aOR, 2.9; 95% CI, 1.1-4.4; Table III).

No evidence of associations were found for modifiable risk factors, including previous prescription of an adrenalin autoinjector, carriage patterns for autoinjectors, or higher risk of accidental allergen exposure through knowingly eating the food to which the student was allergic or eating foods labeled with precautionary allergen labeling, such as "may contain traces of" (data not shown).

DISCUSSION

This is one of the first studies to investigate the population prevalence and characterization of adverse food reactions, including anaphylaxis, and associated risk factors in adolescents. These results show that in a population-based sample of 10- to 14-year-old students with possible IgE-mediated food allergy, almost half (44.4%) reported food-induced allergic reactions in the preceding 12 months, and almost 1 in 10 (9.7%) reported at least 1 reaction with symptoms consistent with anaphylaxis. Reactions occurred most frequently in the home and were triggered most commonly by peanut and tree nuts. Reporting any food allergy reaction in the past 12 months was associated with female sex, having more than 2 food allergies, and having asthma (resolved or current). Nut allergy was the only risk factor found to be associated with reporting anaphylaxis. We observed a trend of increased asthma among those with food allergies reporting anaphylaxis compared with milder reactions. However, our results did not show that those with asthma were at significantly increased risk for severe reactions.

A major strength of this study is that it is both population based and highly detailed in its investigation of food-induced allergic reactions in the adolescent age group. This allowed study of all adolescents with food allergies and those experiencing adverse food reactions and is not limited to clinical samples, such as those presenting to health care facilities for treatment after a reaction or managed by allergy clinics. Although clinical samples have the strength of more robust diagnosis and definition of adverse reactions and anaphylaxis, they risk overrepresenting those with more severe food allergy phenotypes, higher socioeconomic status, and greater access to specialized medical management and education.

TABLE II. Factors predicting adverse food reactions among students with IgE-mediated food allergy in the past 12 months in the SchoolNuts study

	IgE-mediated allergy (no reaction), no. (%)	IgE-mediated allergy (reaction), no. (%)	Crude OR (95% CI)	aOR (95% CI)*	*P* value
Total (n = 547)	304 (55.6)	243 (44.4)			
Sex					
Male (n = 262)	165 (63.0)	97 (37.0)	1.00	1.00	
Female (n = 285)	139 (48.8)	146 (51.2)	1.8 (1.3-2.5)	1.9 (1.3-2.8)	.001
School					
Primary (n = 302)	167 (55.3)	135 (44.7)	1.00	1.00	
Secondary (n = 245)	137 (55.9)	108 (44.1)	0.9 (0.7-1.4)	1.1 (0.7-1.2)	.69
School type					
Government (n = 234)	140 (59.8)	94 (40.2)	1.00	1.00	
Catholic (n = 175)	100 (57.1)	75 (42.9)	1.1 (0.8-1.6)	1.3 (0.8-2.0)	.28
Independent (n = 138)	64 (46.4)	74 (53.6)	1.2 (1.1-2.6)	1.4 (0.8-2.3)	.24
Student's country of birth					
Australia (n = 488)	269 (55.1)	219 (44.9)	1.00	1.00	
Other (n = 58)	42 (58.6)	24 (41.4)	0.9 (0.5-1.5)	0.9 (0.5-1.6)	.69
Asthma details					
No asthma (n = 237)	153 (64.6)	84 (35.4)	1.00	1.00	
Resolved asthma (n = 76)	37 (48.7)	39 (51.3)	1.9 (1.1-3.2)	1.9 (1.1-3.2)	.02
Current asthma (n = 177)	95 (53.7)	82 (33.7)	1.1 (1.0-2.2)	1.7 (1.1-2.6)	.01
Food allergy details					
No. of food allergies					
Single (n = 306)	182 (59.5)	124 (40.5)	1.00	1.00	
Two (n = 138)	76 (58.1)	62 (44.9)	1.9 (0.8-1.8)	1.1 (0.7-1.7)	.73
Multiple (n = 101)	45 (44.6)	56 (55.4)	1.8 (1.1-2.9)	1.9 (1.1-3.1)	.02
Type of food allergy					
Without nut (n = 211)	105 (49.8)	106 (50.2)	1.00	1.00	
With nut (n = 336)	199 (59.2)	137 (40.8)	0.7 (0.5-1.0)	0.7 (0.5-1.0)	.09

Numbers might not add up to total because of missing values.
*All models were adjusted *a priori* for sex, school type, school sector, and student's county of birth. Separate models were fit for the clinical factors of asthma and food allergy details, and food allergy models (number and type of food allergies) were additionally adjusted for asthma because of evidence of confounding.

This study also has some limitations. The definition of anaphylaxis relied on self-reported symptoms involving breathing difficulties, which might have overestimated the true prevalence of anaphylaxis. This definition might also have captured asthmatic episodes that were by chance associated with meals, along with true episodes of food-induced anaphylaxis. However, this is unlikely to have greatly inflated our estimates of anaphylaxis because almost all reactions involving breathing difficulties also had 1 or more symptoms of skin rash, facial swelling, and/or vomiting. In addition, the study population was restricted to those living in urban/suburban areas of Victoria (within 80 km of the central business district), and therefore findings might not be generalizable to rural areas. The study was also underpowered to assess the important issue of fatalities in this age group.

Although parents have a robust appreciation of their child's clinical history, an adolescents' increasing engagement outside the home might reduce the likelihood that parents fully share the adolescent's experience of food allergy. Hence a further strength of SchoolNuts is the inclusion of both adolescent and parent reporting. This has also allowed validation of self-reported responses for food allergy, asthma, and allergic reaction details addressing the limitation of self-reported data. Kappa analysis showed strong correlation between student and parent dyad responses for self-reported food allergy. However, validation of student reports of food reaction details against parent responses was not possible because of differences in questionnaires. Medication or health care contact questions relevant to asthma were not included in the student questionnaire.

A recent systematic review and meta-analysis of food-induced anaphylaxis in patients with food allergy reported a pooled incidence rate of self-reported anaphylaxis in 0- to 19-year-olds of 4.93/100 person years (95% CI, 2.78-8.74) with a range of 0.6 to 8.9/100 person years based on 10 studies.[28] Comparison with our results is difficult because the included articles either involved younger children,[19,20] were based on selected food allergies only,[13-19] or involved high-risk allergy clinic populations.[21] Population-representative studies in adolescence are limited, and comparison is difficult because of differences in population food allergy prevalence and study methodologies.

A large study of 11- to 15-year-old Turkish students (n = 10,096) also recruited adolescents and parents through schools. Using self-reported questionnaires, they reported a slightly higher prevalence of parent-reported food allergy (11.3%) than in our study of adolescent-reported food allergy (9.7%) and a much lower rate of reactions in the past year (3.6% vs 44.5%) in our study. These authors did not report separately on the frequency of anaphylaxis.[29]

Reported only by abstract at this stage, a large birth cohort study in Sweden with a follow-up at 16 years of age reported much lower rates of adverse reactions, with 8.5% of 16-year-olds reporting reactions to food in the past 12 months and 0.8% experiencing anaphylaxis. Overall food allergy prevalence was not reported.[30]

Finally, in a population-based study of 0- to 17-year-olds in Germany, 38% self-reported food-induced allergic reactions.

TABLE III. Factors predicting severe adverse food reactions (anaphylaxis) among students with IgE-mediated food allergy in the past 12 months in the SchoolNuts study

	IgE-mediated reaction (not severe), no. (%)	IgE-mediated reaction (severe), no. (%)	Crude OR (95% CI)	aOR (95% CI)*	*P* value
Total (n = 148)	95 (64.2)	53 (35.8)			
Sex					
Male (n = 63)	37 (58.7)	26 (41.3)	1.00	1.00	
Female (n = 85)	58 (68.2)	27 (31.8)	0.7 (0.3- 1.3)	0.9 (0.4-1.9)	.92
School					
Primary (n = 86)	51 (59.3)	35 (40.7)	1.00	1.00	
Secondary (n = 62)	44 (71.0)	18 (29.0)	0.6 (0.3-1.2)	0.6 (0.3-1.3)	.24
School type					
Government (n = 57)	38 (66.7)	20 (33.3)	1.00	1.00	
Catholic (n = 56)	34 (60.7)	22 (39.3)	1.3 (0.6-2.8)	1.1 (0.5-2.3)	.76
Independent (n = 35)	23 (65.7)	12 (34.3)	1.0 (0.4-2.5)	0.9 (0.4-2.3)	.77
Student's country of birth					
Australia (n = 135)	85 (63.0)	50 (37.0)	1.00	1.00	
Other (n = 13)	10 (76.9)	3 (23.1)	0.5 (0.1-1.9)	0.6 (0.1-2.4)	.45
Asthma details					
No asthma (n = 70)	47 (67.1)	23 (32.9)	1.00	1.00	
Resolved asthma (n = 22)	16 (72.7)	6 (27.3)	0.9 (0.3-2.4)	0.8 (0.3-2.2)	.80
Current asthma (n = 56)	33 (57.1)	24 (45.2)	1.4 (0.7-3.0)	1.6 (0.7-2.6)	.24
Food allergy details					
No. of food allergies					
Single (n = 73)	51 (69.3)	22 (30.7)	1.00	1.00	
Two (n = 33)	21 (63.6)	12 (36.4)	1.3 (0.6-3.1)	1.5 (0.6-2.9)	.39
Multiple (n = 42)	23 (54.8)	19 (45.2)	1.9 (0.9-4.2)	2.1 (0.9-3.8)	.09
Type of food allergy					
Without nut (n = 45)	34 (75.6)	11 (24.4)	1.00	1.00	
With nut (n = 103)	61 (59.2)	42 (40.8)	2.9 (0.9-4.7)	2.9 (1.1-4.4)	.03

Numbers might not add up to total because of missing values.
*All models were adjusted *a priori* for sex, school type, school sector, and student's county of birth. Separate models were fitted for the clinical factors of asthma and food allergy details. and the food allergy models (number of food allergies and type of food allergies) were additionally adjusted for asthma because of evidence of confounding.

However, the majority of the reactions were due to oral allergy syndrome.[31]

Previously, our HealthNuts study has reported the highest rate of challenge-proved food allergy in the world for 1-year-old infants.[1] At almost 10%, a very high burden of food allergy is experienced by parents of allergic infants, with implications for the Australian health care system. Self-reported food allergy prevalence of 6.6% in young adolescents within the SchoolNuts study supports the natural history of resolution of food allergy for many children with food allergy. However, the finding that almost half of 10- to 14-year-old adolescents with food allergies experienced reactions in the past 12 months highlights the ongoing burden that food allergy has on subjects, with subsequent effects on their families and health care facilities.

We found that female subjects were more likely to report experiencing any adverse food reaction in the past 12 months but found no significant sex difference associated with anaphylaxis. This is in contrast to observations of increased risk of fatality from anaphylaxis in adolescent male subjects and might reflect the younger age group in our study.[9]

Our data suggest a benefit from the current compulsory training around food allergy that has been in place in the educational sector in Australia (first introduced from 2008 in some states) because fewer reactions occurred in schools compared with restaurants and homes. However, this might simply reflect that more meals are eaten at home versus school or eating out. Moreover, the finding that just over half of reactions occurred in the home highlights the importance of allergen avoidance and education/management strategies targeted at preventing accidental exposure in all environments and not just schools.

We note that less than half (43.2%) of the cases of confirmed anaphylaxis reported adrenalin autoinjector administration, and this worrying result requires further exploration. It is unclear whether this is simply a result of underreporting as a result of retrospective recall or whether this represents true underadministration. However, it is important to note that the well-known risk of anaphylaxis, including reported fatalities in the school environment, has resulted in legislated guidelines for anaphylaxis management for schools in Australia. All Victorian schools with an enrolled student at risk of anaphylaxis are mandated to have an annually updated action plan for each student at risk of anaphylaxis, staff must undertake regular training on anaphylaxis management and autoinjector administration, and generic autoinjectors must be available within the school.

In SchoolNuts one third (32.4%) of adolescents with food allergy reported current asthma. This is much higher than in the Turkish school-based population-representative study, which reported a prevalence of asthma of 12.3% in those with food allergy.[29] We also found those with asthma (both resolved and current asthma) were almost twice as likely to report a food reaction in the past 12 months compared with those with no history of asthma. Surprisingly, those reporting anaphylaxis had more asthma than those reporting milder reactions; however, asthma was not found to be significantly associated with increased risk of anaphylaxis. This trend toward increased

TABLE IV. Details of self-reported food allergy episodes (n = 372) reported by students with possible IgE-mediated food allergy (n = 243) in the past 12 months in the SchoolNuts study

Episodes	All reactions, no. (%) 372	Confirmed anaphylaxis, no. (%)* 44 (11.8)	Unrecognized anaphylaxis, no. (%)† 49 (13.1)	Likely IgE mediated, no. (%)‡ 149 (40.1)	Unlikely IgE mediated, no. (%)§ 42 (11.2)	Reactions that could not be classified, no. (%)‖ 88 (23.7)
Trigger food						
Peanut	66 (17.7)	17 (38.6)	15 (30.6)	30 (20.1)	0	4 (4.5)
Tree nut	49 (13.2)	11 (25.0)	10 (20.4)	[1]23 (15.4)	0	5 (5.7)
Nut, unspecified	17 (4.6)	5 (11.4)	3 (6.1)	9 (6.0)	0	0
Egg	23 (6.2)	2 (4.5)	2 (4.1)	17 (11.4)	1 (2.4)	1 (1.1)
Milk	56 (15.1)	5 (11.4)	2 (4.1)	19 (12.8)	22 (52.4)	8 (9.1)
Shellfish and fish	16 (4.3)	2 (4.5)	5 (10.2)	8 (5.4)	0	1 (1.1)
Sesame	1 (0.3)	0	1 (2.0)	0	0	0
Kiwifruit	12 (3.2)	0	1 (2.0)	11 (7.4)	0	0
Pine nut	2 (0.5)	0	2 (4.1)	0	0	0
Soy	5 (1.3)	1 (2.3)	1 (2.0)	2 (1.3)	0	1 (1.1)
Wheat	11 (2.9)	0	1 (2.0)	0	9 (21.4)	1 (1.1)
Other foods	48 (12.9)	0	4 (8.2)	24 (16.1)	8 (19.0)	12 (13.6)
Food not reported	66 (17.7)	1 (2.3)	2 (4.1)	6 (4.0)	2 (4.8)	55 (62.5)
Symptoms¶						
Skin rash	150 (40.3)	26 (59.1)	26 (53.1)	87 (58.4)	3 (7.1)	8 (9.1)
Facial swelling	96 (25.8)	33 (75.0)	19 (38.8)	41 (27.5)	0	3 (3.4)
Vomiting	61 (16.4)	12 (27.3)	9 (18.4)	31 (20.8)	5 (11.9)	4 (4.5)
Diarrhea	32 (8.6)	2 (4.5)	3 (6.1)	14 (9.4)	12 (28.6)	1 (1.1)
Breathing difficulty	131 (35.2)	44 (100.0)#	49 (100.0)**	34 (22.8)	0	1 (1.1)
Other	78 (21.0)	9 (20.4)	12 (24.5)	28 (18.8)	26 (61.9)	3 (3.4)
NA	73 (19.6)	0	0	3 (2.0)	2 (4.8)	68 (77.3)
Location						
Home	170 (45.7)	17 (38.6)	20 (40.1)	87 (58.4)	25 (59.5)	21 (23.9)
Restaurant	33 (8.9)	7 (15.9)	9 (18.4)	13 (8.7)	3 (7.1)	1 (1.1)
School	27 (7.3)	4 (9.1)	4 (8.2)	15 (10.1)	3 (7.1)	1 (1.1)
Friend's house	21 (5.6)	4 (9.1)	3 (6.1)	10 (6.7)	1 (2.4)	3 (3.4)
Other	50 (13.4)	12 (27.3)	9 (18.4)	20 (13.4)	8 (19.0)	1 (1.1)
NA	71 (19.1)	0	4 (8.2)	4 (2.1)	2 (4.8)	61 (69.3)
EpiPen used						
Yes	26 (6.9)	19 (43.2)	6 (12.2)	1 (0.7)	0	0
No	319 (85.8)	21 (47.7)	40 (81.6)	145 (97.3)	38 (90.5)	75 (85.2)
Do not know	15 (4.0)	4 (9.1)	1 (2.0)	3 (2.0)	4 (9.5)	3 (3.4)
NA	12 (3.2)	0	2 (4.1)	0	0	10 (11.4)

Tree nut details: *almond × 1, pistachio × 2, hazelnut × 1, cashew × 2, walnut × 3, Brazil nut × 1, and macadamia × 1; †walnut × 3, cashew × 6, and almond × 1; ‡cashew × 11, walnut × 5, hazelnut × 6, almond × 1, and macadamia × 1; and ‖hazelnut × 1, and walnut × 4. *Other food details*: †pineapple × 1, eggplant × 2, kiwifruit × 5, and confectionary × 1; ‡watermelon × 1, chocolate × 2, strawberry × 3, celery × 2, citrus fruit × 3, avocado × 1, pineapple × 4, tomato × 3, mango × 1, mushroom × 1, banana × 1, eggplant × 1, and apple × 1; §cauliflower × 1, pork × 1, food coloring × 2, citrus × 1, spring roll × 1, banana × 1, fructose × 1, and lactose × 1; and ‖chocolate × 4, strawberry × 1, kiwifruit × 2, cake × 1, beef × 1, rice × 1, blueberry × 1, and garlic × 1.

NA, Not available.

¶Numbers do not add up to total because each episode could have multiple symptoms recorded.

#Forty-one of 44 episodes of confirmed anaphylaxis reported breathing difficulties with 1 or more symptoms of skin rash, facial swelling, and/or vomiting.

**Forty-two of 49 episodes of "unrecognized anaphylaxis" reported breathing difficulties with 1 or more symptoms of skin rash, facial swelling, and/or vomiting.

association of asthma with increased severity of reactions is supported by case series that report up to 50% of children (0-18 years) with reactions severe enough to present to an emergency department having coexistent asthma[32] and almost all fatalities from food-induced anaphylaxis reported to have coexisting asthma.[10,11] Our study might have been underpowered to observe an association for asthma and anaphylaxis, and we did not capture or report on fatality data. Asthma as a risk factor for food-induced anaphylaxis requires further investigation.

Peanut and tree nuts were the most common food allergies reported in our study, including among students with a history of anaphylaxis, which is consistent with other studies.[24,32-34] Among those reporting reactions, nut allergy increased the risk 3-fold of having a reaction consistent with anaphylaxis when compared with those without nut allergy. Yet among those with IgE-mediated food allergy, nut allergy was associated with decreased risk of having a reaction in the past 12 months. This apparently paradoxical result might be the result of widespread awareness of the potential severity of nut allergy and adoption of strategies to assist in nut avoidance, such as food policies banning nuts. It might also be easier to avoid nut ingestion than other more ubiquitous food allergens, such as egg or milk. However, the association of nut allergy with more severe reactions might be that once accidental exposure to an allergen occurs, nuts elicit more severe reactions than exposure to other allergens, such as milk and egg, which are often tolerated in a baked or processed form.[35,36]

Having more than 2 food allergies doubled the risk of a food allergy reaction compared with those with a single food allergy, and there was a trend toward higher risk of reactions consistent with anaphylaxis among those with more than 2 food allergies compared with those with a single food allergy. This might reflect that the chance of accidental exposure is higher if multiple food allergens need to be avoided. Additionally, this might be indicative of a higher-risk subset of adolescents with multiple food allergies because of coexisting allergies to the more common later-onset allergies (fish/shellfish, nuts, or both) together with persistent milk allergy, egg allergy, or both. Milk and egg allergy have previously been thought to resolve by early school age, but recent data suggest increasing persistence into adolescence, with persistent allergy associated with more severe reactions to milk and egg.[35,36] Turner's report of anaphylaxis in adolescents from a tertiary clinical center in London[37] showed that the majority of those with recent severe anaphylactic reactions had been triggered by exposure to cow's milk protein in the context of persistent cow's milk allergy from earlier childhood. Although the study by Turner[37] did not report the presence of other coexisting food allergies, adolescents with multiple food allergies, including unresolved milk allergy, egg allergy, or both, might represent a higher-risk phenotype for anaphylaxis that has not been sufficiently considered to date.

In conclusion, within a population-based sample of 10- to 14-year-old adolescents with food allergy in Melbourne, Australia, there was an alarmingly high rate of food-induced allergic reactions, including anaphylaxis. Reactions occurred most frequently in the home and were most commonly triggered by peanut and tree nuts. Those with asthma and more than 2 food allergies were at greatest risk for reactions, but those with nut allergies were most at risk of reactions consistent with anaphylaxis.

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Clinical implications: Adolescents are at highest risk of death from food-induced anaphylaxis. This study offers population-representative data on the frequency, characteristics, and associated risk factors for adverse food reactions among adolescents. It highlights also the alarming frequency of adverse food reactions among adolescents and the need for specific management and education strategies aimed at allergen avoidance in this high-risk age group.

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6. THE AUSTRALIAN PREVALENCE AND DEVELOPMENT OF TREE NUT ALLERGY

6.1 Introduction

This chapter is presented in the form of a manuscript that was published in the Journal of Clinical Immunology and Allergy in October 2018. This research was also presented as an oral poster at the European Allergy and Clinical Immunology (EAACI) Paediatric Meeting (PAAM) in London October 2017.

Studies in children of tree nut allergy prevalence based on OFC outcomes have been limited, with no Australian studies to date. Development of tree nut allergy in childhood is also understudied. The first presentation for children with food allergy is often via reactions to milk, peanut and/or egg in infancy and early sensitisation to tree nuts is reported in 19% of those with food allergies by 2 years of age.[169] How this early sensitisation relates to the subsequent development of tree nut allergy is uncertain and has not been studied at the population level nor systematically assessed using protocolised challenges. There is a current clinical dilemma as to what should be done regarding tree nut allergy testing and introduction advice for those with either peanut allergy or other forms of food allergy in infancy

Therefore, this manuscript addresses the following research questions:

1. What is the prevalence of tree nut allergy in Australian children aged 1 and 6 years of age?
    d) What is the prevalence of parent-reported tree nut allergy in the HealthNuts study at age 1 year?
    e) What is the prevalence of challenge-confirmed tree nut allergy in the HealthNuts study at age 6 years?

f) What are the most common individual tree nut allergies in children at age 6 years?

2. What proportion of those with challenge-confirmed food allergy at age one year are tree nut sensitised?
3. What is the relationship between tree nut sensitisation at age one year and tree nut allergy at age 6 years?
4. What is the relationship between food allergy type at age one year and tree nut allergy at age 6 years?
5. What is the frequency of co-allergy to peanut and other nuts at age 6 years?

6.2 The Manuscript

Patterns of tree nut sensitization and allergy in the first 6 years of life in a population-based cohort

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Background: Longitudinal population-based data regarding tree nut allergy are limited.
Objectives: We sought to determine the population prevalence of tree nut allergy at age 6 years and explore the relationship between egg and peanut allergy at age 1 year and development of tree nut allergy at age 6 years.
Methods: A population-based sample of 5276 children was recruited at age 1 year and followed up at age 6 years. At age 1 year, allergies to egg and peanut were determined by means of oral food challenge, and parents reported their child's history of reaction to tree nuts. Challenge-confirmed tree nut allergy was assessed at age 6 years.
Results: At age 1 year, the prevalence of parent-reported tree nut allergy was 0.1% (95% CI, 0.04% to 0.2%). Only 18.5% of infants had consumed tree nuts in the first year of life. At age 6 years, challenge-confirmed tree nut allergy prevalence was 3.3% (95% CI, 2.8% to 4.0%), with cashew the most common (2.7%; 95% CI, 2.2% to 3.3%). Of children with peanut allergy only at age 1 year, 27% (95% CI, 16.1% to 39.7%) had tree nut allergy at age 6 years compared with 14% (95% CI, 10.4% to 17.9%) of those with egg allergy only and 37% (95% CI, 27.2% to 47.4%) of those with both peanut and egg allergy.
Conclusions: Tree nut allergy is uncommon in the first year of life, likely because of limited tree nut consumption. At age 6 years, tree nut allergy prevalence is similar to peanut allergy prevalence. More than a third of children with both peanut and egg allergy in infancy have tree nut allergy at age 6 years. Understanding how to prevent tree nut allergy should be an urgent priority for future research. (J Allergy Clin Immunol 2018;■■■:■■■-■■■.)

Key words: *Food allergy, sensitization, tree nut allergy, prevalence, population*

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Tree nut allergies are usually lifelong and together with peanut allergy are the most common cause of food-induced anaphylaxis and related fatalities.[1,2] Unlike peanut allergy, population-based data regarding tree nut allergy are limited.

Recently, we reported that 2.3% of 10- to 14-year-old Australian children had clinic-confirmed tree nut allergy[3]; however, prevalence estimates using challenge confirmation remain limited at less than 10 years of age. To date, studies in younger children of challenge-confirmed food allergy outcomes have been limited to regions reporting very low overall rates of food allergy (<1%)[4-6] or where low numbers of tree nut food challenges were performed.[7]

Development of tree nut allergy in childhood is also understudied, with little understanding of the role of early tree nut sensitization and food allergy type. The first presentation for children with food allergy is often through reactions to peanut or egg in infancy. Children with peanut allergy are thought to be at increased risk of tree nut allergies, with around 30% of pediatric patients presenting with peanut allergy reported to have allergies to tree nuts.[8-12] To date, no study has explored this clinical observation at the population level nor systematically assessed the association using protocolized challenges. The current clinical dilemma remains: What should be done regarding tree nut allergy testing and introduction advice for those with either peanut allergy or other forms of food allergy in infancy?

The objectives of this study were to estimate the population prevalence of clinic-confirmed tree nut allergies during the first 6 years of life and describe the patterns of coexisting allergies to

Abbreviations used
OFC: Oral food challenge
SPT: Skin prick test

peanut and other tree nuts. We also aimed to explore the relationship between food allergy at age 1 year and the subsequent development of tree nut allergy at age 6 years.

METHODS

The HealthNuts study is a population-representative longitudinal study of 5276 children recruited at age 1 year and followed up to age 6 years. The study methods have been described in detail previously.[13-15] To summarize, between 2007 and 2011, 5276 infants aged between 11 and 15 months were recruited from immunization clinics around Melbourne, Australia. At recruitment, all infants underwent skin prick tests (SPTs) to egg, peanut, and sesame, and parents completed a questionnaire. The first half of the cohort also had SPTs to shrimp, whereas the second half of the cohort had SPTs to cow's milk.[13]

SPTs were performed with single-tine lancets (Stallergenes, Antony, France) on the infant's back using allergens from ALK-Abelló (Madrid, Spain), along with a positive control (10 mg/mL histamine) and a negative control (saline). Wheal size was measured after 15 minutes and calculated by subtracting the negative control from the average of the longest diameter and the diameter perpendicular to it. Parental report of the history of allergic reactions in the first year of life was determined by means of questionnaire.

All children who showed any reaction on SPTs (wheal size ≥1 mm), as well as a random sample of those with negative SPT responses, were invited to attend a study clinic at the Royal Children's Hospital for repeat SPTs and oral food challenges (OFCs). At age 1 year, OFCs were performed only for egg, peanut, and sesame.

Those attending the clinic had additional SPTs performed for tree nuts (almond, cashew, and hazelnut). No OFCs were performed for tree nuts at age 1 year. For those with negative SPT responses to tree nuts, home introduction was advised. For those with positive SPT responses, avoidance was recommended.

Follow-up methods at 4 and 6 years of age have been described previously.[14] To summarize, at age 4 years, all participants were followed up by means of questionnaire, and those who reported a new food-induced allergic reaction and those who had any food allergy at age 1 year were invited for clinic assessment that included SPTs and OFCs.

At age 6 years, the entire cohort (n = 5276) was invited to participate in questionnaire and SPT assessment. Questionnaires were mailed to all participants, capturing demographic details, history of food allergy and new food reactions, common allergen exposure information, and history of asthma/wheeze and eczema. All participants were invited for an allergy/health assessment that included SPTs to a predetermined panel of 8 foods (milk, egg, peanut, wheat, sesame, cashew, almond, and hazelnut) and was conducted either in the child's home or at the Royal Children's Hospital. Those with positive SPT responses (>1 mm) or parent-reported reactions to foods consistent with an IgE-mediated allergy were invited for a clinic appointment with a specialist allergy nurse, and OFCs were conducted when indicated by using a standardized protocol (see Fig E1 in this article's Online Repository at www.jacionline.org). Those who were sensitized to almond, cashew, or hazelnut had additional tree nut SPTs performed to all nontolerated tree nuts at the second clinic visit, including Brazil nut, macadamia, pecan, pistachio, and walnut. OFCs were conducted, as previously described,[16] and results were deemed positive if they met at least 1 of the following predefined criteria: (1) 3 or more concurrent noncontact urticarias lasting at least 5 minutes; (2) severe persistent vomiting; (3) perioral or periorbital angioedema; or (4) anaphylaxis (evidence of circulatory or respiratory involvement) within 2 hours of the last challenge dose in the presence of IgE sensitization.

Definitions

Age 1 year. *Sensitized tolerant to egg, peanut, or sesame* was defined as an SPT response of 2 mm or greater and a negative OFC result to that food.

Egg, peanut, or sesame allergy was defined as an SPT response of 2 mm or greater and a positive OFC result to that food.

Milk allergy was defined as an SPT response of 2 mm or greater and a history of reaction consistent with IgE-mediated food allergy.

Parent-reported tree nut allergy was defined as parental report of a reaction consistent with IgE-mediated food allergy to 1 or more tree nuts (any acute onset of skin rash, facial swelling, vomiting, or breathing difficulties within 1 hour of food ingestion).

Tree nut sensitization was defined as an SPT response of 3 mm or greater to almond, cashew, or hazelnut.

Tree nut tolerance was defined as a history of tolerance on ingestion or a negative SPT response when undertaken.

Age 6 years. *Tree nut sensitization* was defined as an SPT response of 3 mm or greater to almond, Brazil nut, cashew, hazelnut, macadamia, pecan, pistachio, or walnut.

Definite tree nut allergy was defined as any of the following: (1) positive OFC result and IgE sensitization at age 6 years; (2) history of objective reaction in the past 12 months consistent with HealthNuts OFC stopping criteria after definite exposure to the food of interest and evidence of IgE sensitization at 6 years; or (3) a positive OFC result at age 4 years and SPT response of 8 mm or greater at 6 years of age.

Probable tree nut allergy was defined as any of the following: (1) SPT response of 8 mm or greater but no age 4 years OFC or recent reaction history and no known tolerance or (2) SPT response of 3 to 7 mm at age 6 years and one of (A) positive OFC result at age 4 years, (B) history of objective reaction more than 12 months ago consistent with HealthNuts OFC stopping criteria after definite exposure to the food of interest, or (C) parental report of food avoidance because of allergy.

Tree nut tolerant was defined as any of the following: (1) negative OFC result; (2) SPT response of 0 to 2 mm; (3) SPT response of 3 to 7 mm and parent-reported ingestion history (eaten >1 time since age 4 years); or (4) no reaction since age 4 years and no parental report of food avoidance.

Statistical methods

The prevalence of tree nut allergy was calculated among those who completed an allergy assessment at age 6 years (n = 3232) and limited to the tree nuts included in the screening SPT panel (cashew, hazelnut, and almond). Those with negative SPT responses were deemed tree nut tolerant.

To assess whether these estimates were influenced by characteristics that were associated with participation in allergy assessment at age 6 years, we adjusted for differences in risk factors between participants with and without missing data at age 6 years by using the inverse probability weighting method.[17] This reweighting was used to reflect the distribution of risk factors among those approached but did not participate versus those who underwent a full allergy assessment. Weights were the inverse of the predicted probability of participation obtained after fitting a logistic regression model including covariate risk factors that were associated with completing an assessment rather than questionnaire only or nonparticipation (socioeconomic status, family history of allergy, parent country of birth, and whether the child had challenge-confirmed food allergy or eczema at age 1 year). This generated a propensity score for each participant.[17]

As a sensitivity analysis, tree nut allergy prevalence was calculated as the number of children with tree nut allergy (definite or probable tree nut allergy) to 1 or more tree nuts expressed as a proportion of the entire HealthNuts cohort (n = 5276). It was assumed that those with no SPT data and no known food allergy were tree nut tolerant. This provides the most conservative prevalence estimate. All prevalence estimates are reported as the observed proportion with 95% CIs calculated by using the normal approximation to the binomial distribution.

The proportion of those with nut allergies (definite and probable allergy) with coallergy to other tree nuts was calculated. As a sensitivity analysis, this proportion has also been calculated with all those sensitized at between 3 and

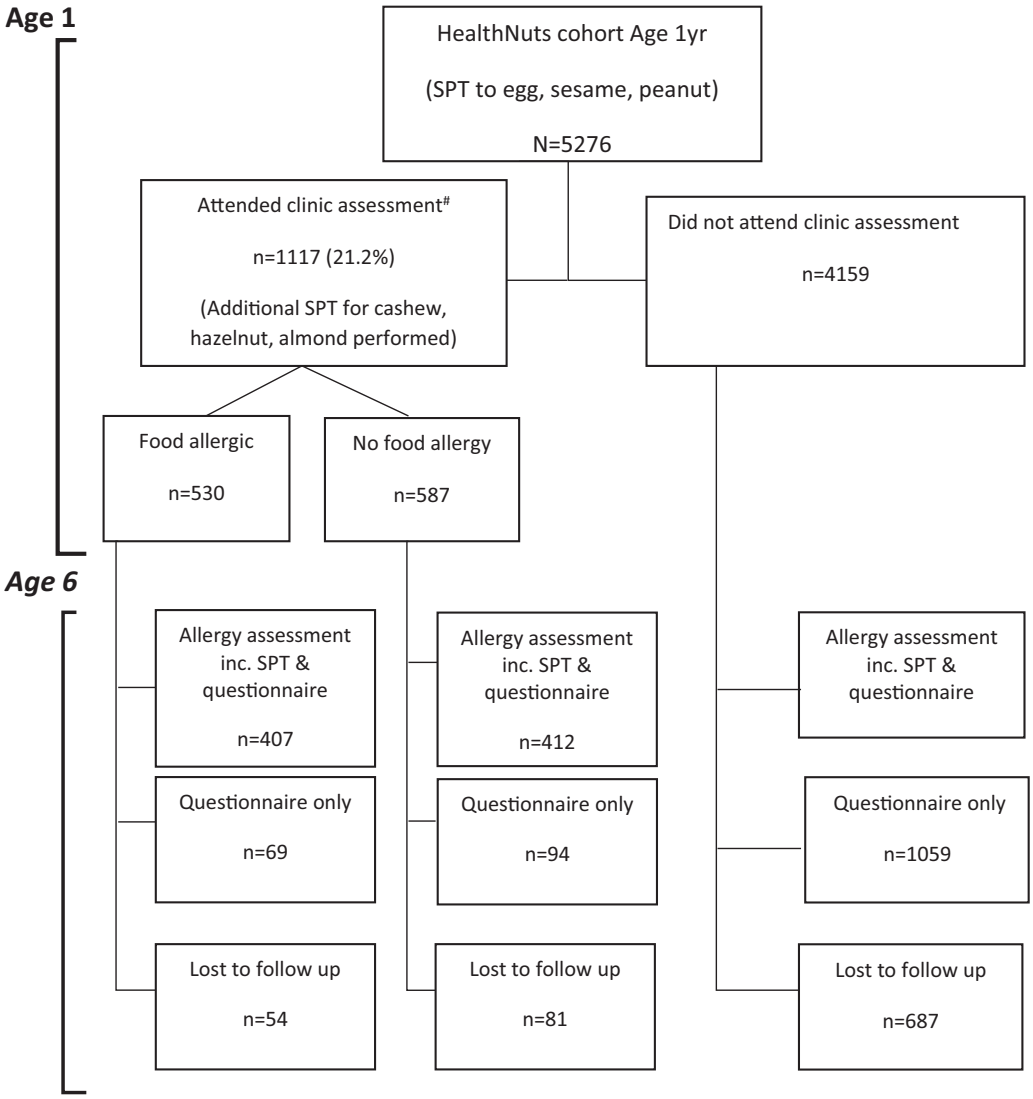

FIG 1. Overview of HealthNuts study participation at age 1 and 6 years.

7 mm who did not have an OFC performed included as allergic. Both calculations have been reported.

Ethics

Ethics approval was obtained for the HealthNuts study from the Victorian State Government Office for Children (reference no. CDF/07/492), the Victorian State Government Department of Human Services (reference no. 10/07), and the Royal Children's Hospital Human Research Ethics Committee (reference no. 27047).

RESULTS

An overview of the HealthNuts study is provided in Fig 1. A total of 5276 one-year-old infants participated in the HealthNuts study at age 1 year (74% participation). Of these, 924 had positive SPT responses to egg, peanut, sesame, or shrimp/cow's milk; attended the OFC clinic; and had SPTs to tree nuts (cashew, almond, and hazelnut). An additional 193 control subjects with negative results attended the clinic for SPTs. At age 1 year, 530 participants were given a diagnosis of OFC-confirmed food allergy to egg, peanut, or sesame.

At age 6 years, 84.4% of the cohort participated in follow-up, with 61.3% (n = 3232) completing both a questionnaire and allergy assessment, including tree nut SPTs, and 23.1% (n = 1222) completing a questionnaire only. Participants with a family history of allergy and children with eczema or food allergy at age 1 year were more likely to participate in the follow-up at age 6 years (Table I).

Parent-reported tree nut allergy at age 1 year

At age 1 year, 6 parents reported a reaction to tree nut consistent with an IgE-mediated food reaction, representing an overall prevalence of 0.1% (95% CI, 0.04% to 0.2%) among the whole cohort of 5276 infants. The low prevalence of reactions might be due to the low consumption of tree nuts in the first year of life, with only 18.5% of parents reporting that their infants had consumed any tree nut by age 1 year.

TABLE I. Demographic and clinical characteristics of the HealthNuts study cohort by participation status at age 6 years

	Assessment cohort (n = 3232 [%])	Questionnaire only (n = 1222 [%])	Did not participate (n = 822 [%])	*P* value†
Sex (male)	52	50	48	.187
SEIFA*				.003
Quintile 1 (least disadvantaged)	18.8	20.3	25.3	
Quintile 2	20.0	20.3	19.2	
Quintile 3	21.9	20.3	18.2	
Quintile 4	19.7	19.5	17.8	
Quintile 5	19.6	19.4	19.4	
Parents' country of birth				<.001
Both Australian	61.8	59.8	49.4	
One East Asian	4.4	4.5	4.1	
Both East Asian	5.8	5.0	9.8	
Other	28.0	30.7	36.6	
Mode of delivery				.601
Vaginal	66.4	67.9	66.1	
Cesarean	33.6	32.1	33.9	
Premature birth	6.2	6.0	5.4	.470
Any siblings	49.9	49.1	50.1	.450
Family history of any allergy	72	66	63	<.001
Family history of food allergy	12	10	9	.007
Family history of asthma	33	27	29	<.001
Family history of eczema	32	28	26	.001
Eczema diagnosis by age 1 y	29	21	24	<.001
Wheeze by age 1 y	18	15	21	.010
Any food allergy at age 1 y	13	7	6	<.001

*Socioeconomic status was assigned on the basis of home postcode by using socioeconomic indexes for areas *(SEIFA)* measures derived from the 2006 Australian census, which accessed relative socioeconomic advantage/disadvantage, economic resources (income, assets, and expenditure) and educational and occupational characteristics.

†χ^2 *P* value refers to any difference between columns 1, 2, and 3.

Tree nut sensitization at age 1 year among those with food allergy

Among those with challenge-confirmed food allergy at age 1 year, 31% (95% CI, 26.6% to 34.7%) were sensitized to 1 or more tree nuts. Tree nut sensitization was less common in infants who were sensitized to 1 or more foods but not allergic (sensitized tolerant; 12% [95% CI, 9.4% to 16.6%]) and in infants with no food sensitization (5.2% [95% CI, 2.7% to 9.3%; Table II).

Tree nut sensitization was more common in infants with both peanut and egg allergy (48.4% [95% CI, 38% to 58.9%]) compared with that in infants with single egg or peanut allergies (23.6% [95% CI, 19.7% to 28.9%] and 33.3% [95% CI, 21.7% to 46.7%], respectively; Table II).

Tree nut allergy at age 6 years

At age 6 years, 234 children were sensitized, and 154 children were allergic to 1 or more tree nuts. Of those with an SPT to tree nuts at age 6 years (n = 3232), the observed prevalence of tree nut sensitization was 7.3% (95% CI, 6.4% to 8.3%), and that of tree nut allergy was 4.3% (95% CI, 3.8% to 5.2%; see Table E3 in this article's Online Repository at www.jacionline.org). After reweighting this estimate for differences in characteristics of subjects who did and did not participate in assessments at age 6 years, the weighted prevalence of tree nut allergy was 3.3% (95% CI, 2.8% to 4.0%). Cashew was the most common tree nut allergy (2.7% [95% CI, 2.2% to 3.3%), followed by hazelnut (0.9% [95% CI, 0.7% to 1.3%) and then almond (0.3% [95% CI, 0.1% to 0.5%]; Fig 2).

All other individual tree nut allergies were diagnosed in less than 1.0% of participants (pistachio, n = 50; walnut, n = 28; macadamia, n = 12; pecan, n = 8; and Brazil nut, n = 5; Table III).

Among the whole cohort of 5276 children, the prevalence of tree nut sensitization was 4.4% (95% CI, 3.9% to 5.0%) and that of tree nut allergy was 3.1% (95% CI, 2.6% to 3.6%). This estimate is likely to be conservative because it assumes that all children who were lost to follow-up did not have tree nut allergy. Collectively, tree nut allergy prevalence (3.3%) was similar to peanut allergy prevalence (2.8% [95% CI, 2.4% to 3.3%). A summary of the SPT and OFC outcomes at age 6 years is included in Fig E2 in this article's Online Repository at www.jacionline.org.

Coallergy patterns among tree nuts

Coallergy patterns among those with any nut allergy are outlined in Table III. Of the 154 children with any tree nut allergy at age 6 years, 42.9% (n = 66) also had peanut allergy at age 6 years. Eighty-four (52.2%) were allergic to only 1 tree nut, 26.7% to 2 tree nuts, 12.4% to 3 tree nuts, and 8.7% to more than 3 tree nuts. Of those with cashew allergy, 36% had coexisting pistachio allergy, and if all those with pistachio sensitization results of between 3 and 7 mm who did not have an OFC were deemed allergic, this increased to 46%.

Of the 147 children with peanut allergy at age 6 years, 45% also had 1 or more tree nut allergies. The most common tree nut coallergy for those with peanut allergy at age 6 years was to cashew (36.7%; Table III).

Tree nut allergy at age 6 years among children with egg or peanut allergy in infancy

Of those with peanut allergy only at age 1 year, 27% had tree nut allergy at age 6 years compared with 14% of those with egg allergy only. A greater proportion (37%) of those with both peanut and egg allergy at age 1 year had tree nut allergy at age 6 years (Fig 3).

Tree nut sensitization at age 1 year and development of tree nut allergy at age 6 years

Of 168 children who were sensitized to cashew at age 1 year, 39% had cashew allergy, and 35% were cashew tolerant at age 6 years (Table IV). Of those sensitized to almond at age 1 year (n = 87), 11% had almond allergy, and 59% were almond tolerant, whereas of those sensitized to hazelnut (n = 72), 19% had hazelnut allergy, and 53% were hazelnut tolerant at age 6 years.

DISCUSSION

This is the first population-based longitudinal study to characterize food challenge–confirmed tree nut allergy in

TABLE II. Cosensitization to tree nuts at age 1 year among the HealthNuts clinic cohort (n = 1117) stratified by food allergy status

Age 1 y food allergy status	Cashew, almond, or hazelnut sensitized (% [95% CI])	Cashew sensitized (% [95% CI])	Almond sensitized (% [95% CI])	Hazelnut sensitized (% [95% CI])
No food allergy/not sensitized (n = 193)	5.2 (2.7-9.3)	3.9 (1.7-7.4)	1.5 (0.3-4.1)	0.5 (0.1-2.7)
Sensitized tolerant* (n = 384)	12.0 (9.4-16.6)	9.4 (6.36-12.9)	3.6 (1.9-6.1)	3.3 (1.7-5.7)
Egg allergy only (n = 347)	23.6 (19.7-28.9)	17.7 (13.8-22.0)	8.9 (6.2-12.4)	7.5 (5-10.8)
Peanut allergy only (n = 60)	33.3 (21.7-46.7)	23.3 (13.4-36.1)	13.3 (5.9-24.6)	8.3 (2.7-18.4)
Peanut and egg allergy† (n = 96)	48.4 (38-58.9)	38.9 (29.1-49.5)	24.2 (16.0-34.1)	20 (12.5-29.5)
Any other allergies‡ (n = 37)	44.4 (27.9-61.9)	38.9 (23.1-56.5)	30.5 (16.3-48.1)	25 (12.1-42.2)

**Sensitized tolerant* is defined as an SPT response of 2 mm or greater to egg, peanut, or sesame and a negative OFC result to that food.
†Allergic to both peanut and egg, irrespective of other food allergies.
‡All other allergies: single milk, 8; single sesame, 5; milk or sesame with either egg or peanut, 23.

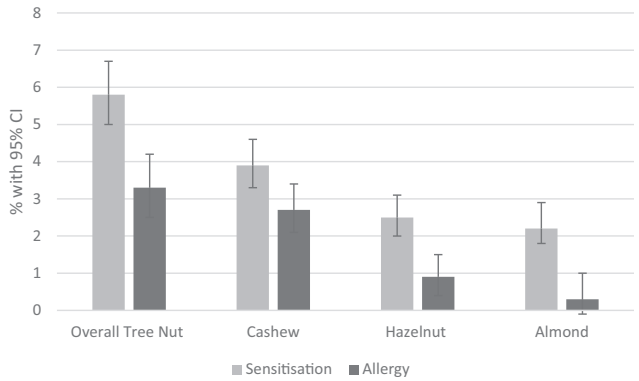

FIG 2. Tree nut sensitization and allergy prevalence at age 6 years.

childhood. It is also the first population-based study to characterize the development of tree nut allergy among children with infantile egg and peanut allergy. We found the prevalence of tree nut allergy at age 6 years (3.3%) to be as common as that of peanut allergy (2.8%), with cashew the most common individual tree nut allergy (2.7%). At age 1 year, 41% of those with challenge-confirmed allergy to egg or peanut were already sensitized to 1 or more tree nuts, and those with both allergies were at greatest risk for tree nut sensitization. Cashew was the most common tree nut sensitization at age 1 year, and around 40% of children sensitized to cashew at age 1 year were allergic to cashew at 6 years of age. Almost half (48%) of the children with both peanut and egg allergy at age 1 year had tree nut allergy at age 6 years.

The strengths of the HealthNuts study are the large population-representative sample, high participation fraction, and good internal and external validity.[13] The follow-up at age 6 years had high cohort retention (>80%). Most tree nut allergy outcomes were clinically confirmed based on objective criteria, with outcomes based on predetermined objective stopping criteria, and the remainder were determined based on large SPT wheal sizes and a history of reported objective adverse reactions consistent with IgE-mediated food allergies.

Limitations included use of open OFCs rather than double-blind, placebo-controlled OFCs, although only objective criteria were used to define a positive challenge result, and nurses were blind to SPT wheal size and history of previous reaction. Not all study participants had SPT or nut consumption data available at age 6 years. Almond, cashew, and hazelnut had more complete screening, and there are limited SPT and OFC data for the additional tree nuts. Most of those deemed allergic to Brazil nut, macadamia nut, pecan, pistachio, and walnut are based on high-level sensitization (SPT response, >8 mm) but not OFC results, and many of those with midrange sensitization (3-7 mm) did not have OFCs performed. This limits the coallergy patterns reported and is an important factor to consider for tree nut allergy studies in the future. In addition, there is also likely to be an allergic bias in participation and follow-up at age 6 years. Therefore we have reported a range of more and less conservative observed prevalence estimates and a population prevalence estimate for tree nut allergy reweighted for factors that were associated with participation at age 6 years.

There are few prevalence studies reporting challenge-confirmed outcomes for tree nut allergy. Recently, we undertook a systematic review of tree nut allergy prevalence internationally and found estimates ranged from 0.05% to 4.9%. However, there was significant study heterogeneity resulting from differences in study design and diagnostic methods. We found 7 studies in children reporting challenge-confirmed tree nut outcomes ranging from 0% to 1.4%.[18] Most of these estimates come from countries or regions with low overall food allergy prevalence.[4-6] Our SchoolNuts study of 10,000 children aged 10 to 14 years reported clinic-defined tree nut allergy of 2.3% by using the same OFC protocols as the current HealthNuts study.[3] The slightly lower prevalence in the SchoolNuts study might reflect the older age group studied.

Estimates of allergy prevalence for individual tree nuts are also limited. Our systematic review found high regional variation, with European studies reporting hazelnut as the most common tree nut allergy, largely because of the high rate of birch pollen allergy and its cross-reactivity with hazelnut. In the United Kingdom Brazil nut was reported as the most common tree nut allergy, and walnut and cashew were reported as the most common tree nut allergy in the United States. Here we found cashew to be the most common tree nut sensitization at age 1 year and allergy at age 6 years. Our Australian SchoolNuts study also reported cashew as the most common tree nut allergy in 10- to 14-year-olds and the most common tree nut trigger for food-induced anaphylaxis.[3] The overall nut coallergy prevalence reported in our study (45%) was greater than that of the population-representative SchoolNuts study reporting 30% of 10- to 14-year-olds with peanut allergy having 1 or more tree nut allergies and 30% of those with a tree nut allergy having 1 or more additional tree nut allergies. Several single-center allergy clinics have also reported similar rates of coallergy to 1 or more tree nuts among children with peanut allergy[10,11,19,20]; however, Fleischer et al[2] reported a higher rate of coexisting peanut allergy (68%) among 190

TABLE III. Peanut and tree nut coallergy patterns at age 6 years

	Coexisting peanut allergy (%)	Coexisting almond allergy (%)	Coexisting Brazil nut allergy (%)	Coexisting cashew allergy (%)	Coexisting hazelnut allergy (%)	Coexisting macadamia nut allergy (%)	Coexisting pecan allergy (%)	Coexisting pistachio allergy (%)	Coexisting walnut allergy (%)
Peanut allergic (n = 147)		6.1-14.2	0.7-6.8	36.7-40.8	19.1-21.8	3.4-7.5	2.7-7.5	13.6-18.4	7.5-11.6
Almond allergic (n = 17)	42.8-60		14.3-25.7	78.5-80.0	57.0-64.3	7.1-20.0	14.3-22.9	34.3-35.7	20.0-28.6
Brazil nut allergic (n = 5)	20.0-50	40.0-45.0		60.0-85.0	55.0-60.0	40.0-55.0	20.0-50.0	75.0-80.0-	60.0-60.0
Cashew allergic (n = 121)	35.5-44.8	8.9-20.9	2.4-12.7		22.6-32.1	5.6-12.7	3.2-12.7	36.3-46.2	14.5-19.4
Hazelnut allergic (n = 44)	44.6-54.2	19.1-33.9	6.4-18.6	59.6-72.9		14.9-22.0	10.6-25.4	25.5-37.2	19.1-25.4
Macadamia nut allergic (n = 12)	25.0-47.8	8.3-30.4	16.6-47.8	58.3-74.0	56.5-58.3		16.6-56.5	50.0-73.9	65.2-66.6
Pecan allergic (n = 8)	25.0-40.7	25.0-29.6	12.5-37.0	50.0-63.0	55.6-62.5	25.0-48.1		37.5-59.2	50.0-77.8
Pistachio allergic (n = 50)	30.0-40.3	10.0-17.9	8.0-22.4	90.0-92.5	24.0-32.8	12.0-25.4	6.0-23.9		30.0-38.8
Walnut allergic (n = 28)	28.5-41.5	14.3-17.1	10.7-29.3	63.4-64.3	32.1-36.6	28.6-36.6	14.3-51.2	53.6-63.4	

Screening for tree nut allergy varied for the various tree nuts. Hazelnut, almond, and cashew SPTs were performed for all study participants. The full tree nut SPT panel was only performed for those sensitized to either almond, cashew, or hazelnut. OFCs were limited for some tree nuts, and therefore the figures presented include those who had OFCs and probable allergy as the lower figure and as a sensitivity analysis those who had OFCs and probable food allergy plus those sensitized at 3 to 7 mm and no OFCs performed included as the upper percentage.

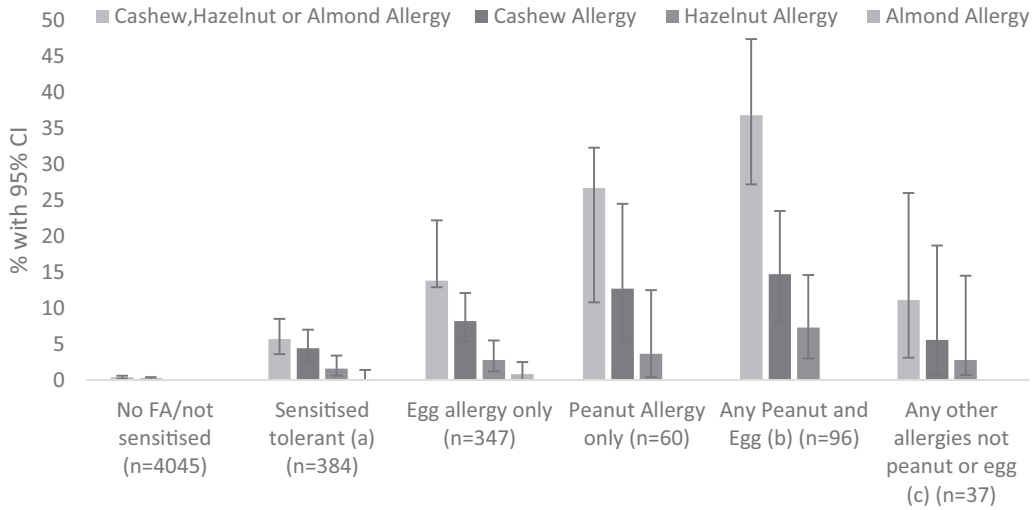

FIG 3. Tree nut allergy (cashew, hazelnut, and almond) at age 6 years by type of food allergy *(FA)* at age 1 year.

children with tree nut allergy in 2005, which might indicate that children with multiple food allergies were overrepresented in their clinics.

Cashew-pistachio and walnut-pecan belong to the same botanical families (Anacardiaceae and Juglandaceae, respectively) and have a reported high degree of serologic cross-reactivity[8,21] and coallergy. The NutCracker study, a single-center prospective cohort study of 83 children with tree nut allergy in Israel, reported two thirds of those with walnut and cashew allergy were also allergic to pecan and pistachio, respectively, whereas all those with pecan and pistachio allergy were allergic to walnut and cashew, respectively.[22] Andorf et al,[23] in a study of 60 selected patients with multifood allergy, reported all those allergic to walnut had coexisting pecan allergy. They reported a unidirectionality of the coallergies, with only two thirds of those patients with walnut and cashew allergy allergic to pecan and pistachio, respectively, suggesting that some allergenic proteins are shared, whereas others are unique to cashew and walnut, resulting in monoallergy.

We found a lower proportion of those with cashew allergy having coexisting pistachio allergy. We did not find pecan-walnut coallergy to be as common as other studies. The differences observed might be due to the limitations of tree nut screening and OFCs in our study because although a smaller study (n = 87), the NutCracker study, did complete OFCs for all sensitized tree nuts, which we were not able to achieve. The observed differences might also to be due to regional differences.

Data on rates of early tree nut sensitization are limited. In 2005, Clark and Ewan[10] reported that by 2 years of age, 19% of those with peanut allergy were sensitized to 1 or more nuts. This was a single-center allergy cohort limited to 47 patients with peanut allergy only. We have reported a markedly higher rate of tree nut sensitization among those with peanut allergy in our population cohort of 33.3%. We also report for the first time a high rate of tree nut sensitization among all those with food allergy at age 1 year and not just peanut allergy. Consideration should be given to identifying this high-risk group of infants in the general population to activate allergy prevention strategies.

This study has found that at 6 years of age, collectively, rates of tree nut allergy are almost as high as those of peanut allergy, with cashew the most common tree nut allergy. Up to half of those with

TABLE IV. Patterns of tree nut sensitization status at age 1 year and sensitization and allergy status at age 6 years (n = 5276)

		Age 6 y tree nut outcome				
				Missing allergy outcome		
Tree nut type	Age 1 y tree nut sensitization status	Allergic, no. (%)	Tolerant, no (%)	3- to 8-mm SPT response and no OFC, no. (%)	No SPT response and unknown tolerance, no. (%)	Missing, no. (%)
Cashew	Positive (n = 168)	66 (39.3)	59 (35.1)	6 (3.6)	2 (1.2)	35 (20.8)
	3-7 mm (n = 110)	26 (19.0)	49 (44.5)	2 (1.8)	2 (1.8)	31 (28.2)
	≥8 mm (n = 58)	40 (70.7)	10 (17.2)	4 (7.0)	0	4 (7.0)
	Negative, <3 mm (n = 947)	38 (4.0)	633 (66.8)	6 (0.6)	7.0 (0.7)	263 (27.8)
	Tree nut SPT not done* (n = 4161)	17 (0.4)	2358 (56.7)	1 (0.02)	39 (1.0)	1746 (42.0)
Almond	Positive (n = 87)	10 (11.5)	51 (58.6)	6 (6.9)	1 (1.1)	19 (21.8)
	3-7 mm (n = 80)	7 (8.8)	49 (61.2)	5 (6.2)	1 (1.2)	17 (21.2)
	≥8 mm (n = 7)	2 (28.6)	2 (28.6)	1 (14.3)	0	2 (28.6)
	Negative, <3 mm (n = 1030)	4 (0.4)	733 (71.0)	8 (0.8)	6 (0.6)	279 (27.1)
	Tree nut SPT not done* (n = 4159)	3 (0.07)	2376 (57.1)	4 (0.1)	29 (0.7)	1747 (42.0)
Hazelnut	Positive (n = 72)	14 (19.4)	38 (52.8)	3 (4.2)	1 (1.4)	16 (22.0)
	3-7 mm (n = 61)	7 (11.5)	35 (57.4)	3 (5.0)	1 (1.6)	15 (24.6)
	≥8 mm (n = 11)	7 (63.6)	3 (27.3)	0	0	1 (10.0)
	Negative, <3 mm (n = 1044)	24 (2.3)	719 (68.9)	10 (1.0)	8 (0.8)	283 (27.0)
	Tree nut SPT not done* (n = 4160)	6 (0.1)	2370 (57.0)	2 (0.1)	35 (0.8)	1747 (42.0)

*Not sensitized to screening foods (egg, peanut, sesame, and shrimp/cow's milk) and therefore did not attend allergy clinic for additional tree nut SPT at 1 year of age.

egg and peanut allergy can be sensitized to tree nuts as early as 1 year of age, and therefore tree nut SPT screening has the potential to impose a significant burden on allergy clinics to confirm allergy status for each tree nut. Evidence that tree nut allergy can be prevented might be required before making recommendations to identify and target children at high risk of tree nut allergy early in life.

Clinical implications: Up to 48% of those with food allergy at age 1 year were found to be tree nut sensitized, and more than a third of those tree nut–sensitized patients had tree nut allergy at age 6 years.

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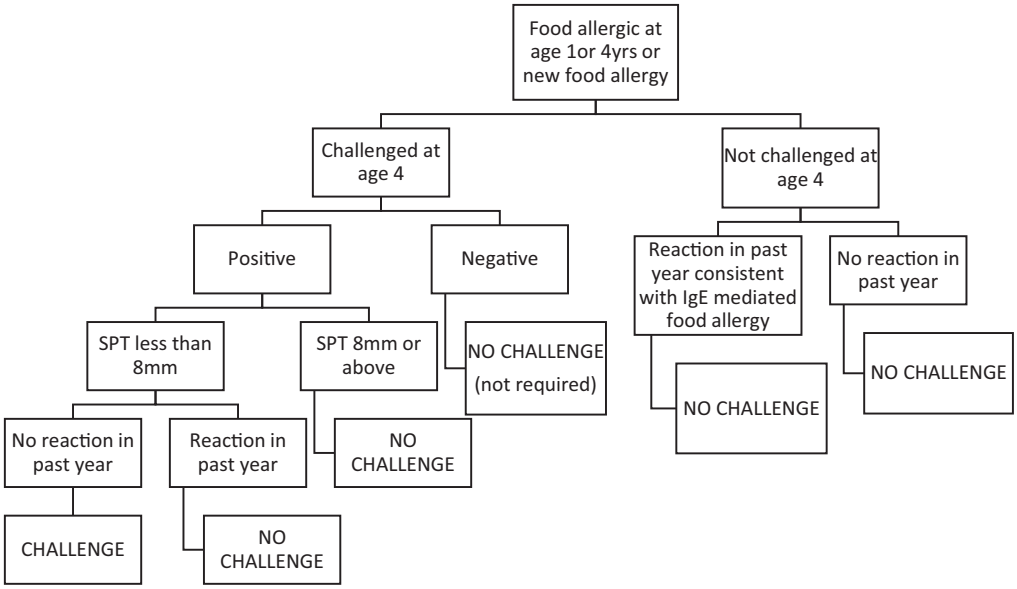

FIG E1. Flow chart of HealthNuts challenge criteria protocol at age 6 years.

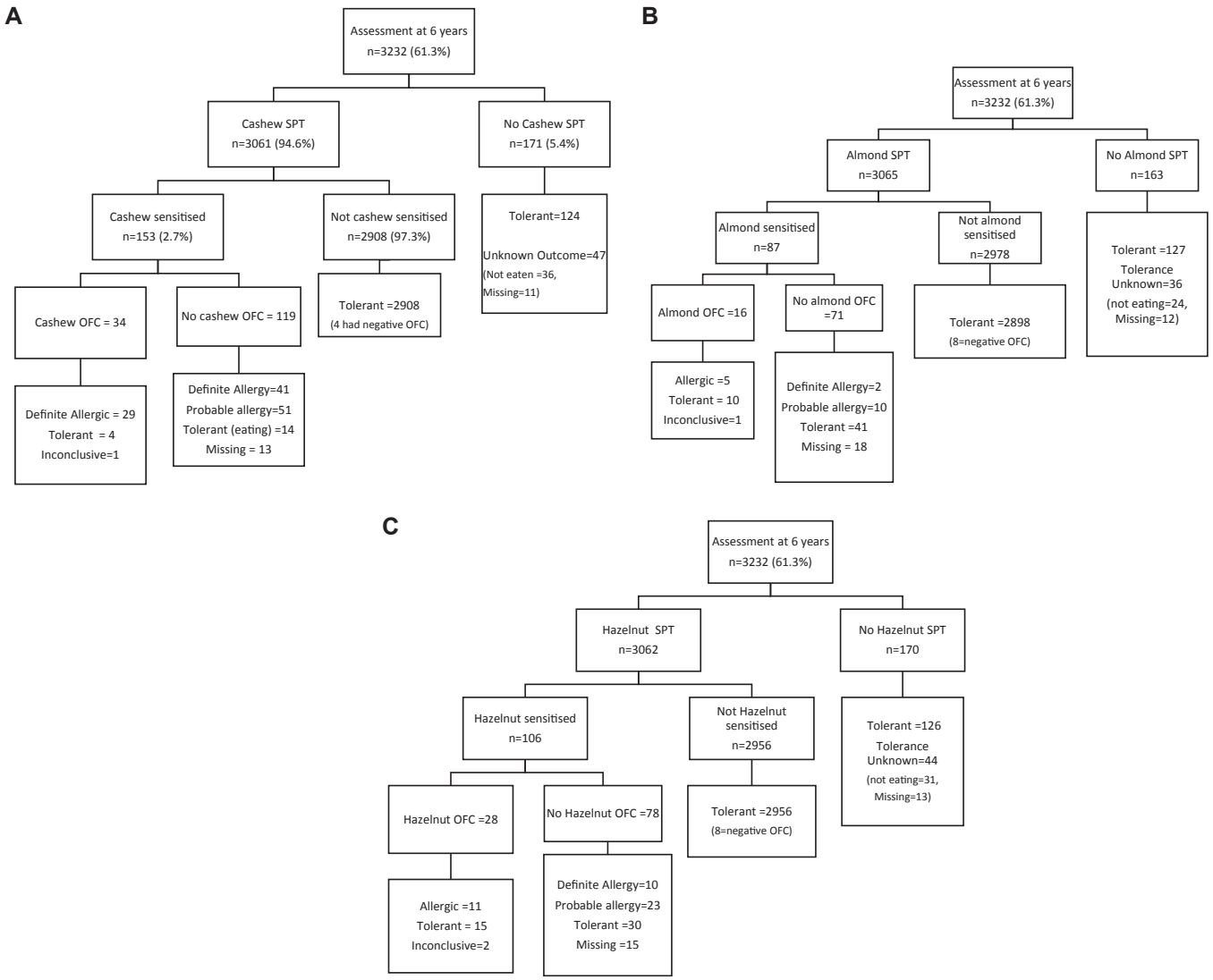

FIG E2. Summary of SPT and OFC outcomes for tree nuts (almond, cashew, and hazelnut) at age 6 years. **A,** *Definite allergy* was defined as any of the following: (1) positive OFC result and IgE sensitized at age 6 years (n = 29); (2) history of objective reaction in the past 12 months consistent with HealthNuts OFC stopping criteria after definite exposure to the food of interest and evidence of IgE sensitization at age 6 years (n = 8); or (3) positive OFC result at age 4 years and SPT response of 8 mm or greater at 6 years of age (n = 33). *Probable allergy* was defined as any of the following: (1) SPT response of 8 mm or greater but no age 4 years OFC or recent reaction history and no known tolerance (n = 48) or (2) SPT response of 3 to 7 mm at age 6 years and one of (A) positive OFC result at age 4 years (n = 3), (B) history of objective reaction more than 12 months ago consistent with HealthNuts OFC stopping criteria after definite exposure to the food of interest (n = 0), or (C) parental report of food avoidance because of allergy (n = 0). *Tolerant* was defined as any of the following: (1) negative OFC result (n = 8); (2) SPT response of 3 to 7 mm and parent-reported ingestion history (eaten >1 time since age years (n = 14 [3]) or not sensitized and no reaction since age 4 years (n = 344 [4]); and (5) no parental report of food avoidance and parent-reported ingestion history (eaten >1 time since age 4 years; n = 2684). **B,** *Definite allergy* is defined as any of the following: (1) positive OFC result and IgE sensitized at age 6 years (n = 5); (2) history of objective reaction in the past 12 months consistent with HealthNuts OFC stopping criteria after definite exposure to the food of interest and evidence of IgE sensitization at age 6 years (n = 2); or (3) positive OFC result at age 4 years and SPT response of 8 mm or greater at 6 years of age (n = 0). *Probable allergy* is defined as any of the following: (1) SPT response of 8 mm or greater but no age 4 years OFCs or recent reaction history and no known tolerance (n = 7) or (2) SPT response of 3 to 7 mm at age 6 years and one of (A) positive OFC result at age 4 years, (B) history of objective reaction more than 12 months ago consistent with HealthNuts OFC stopping criteria after definite exposure to the food of interest, or (C) parental report of food avoidance because of allergy (n = 3). *Tolerant* is defined as any of the following: (1) negative OFC result (n = 18); (2) SPT response of 3 to 7 mm and parent-reported ingestion history (eaten >1 time since age 4 years; n = 41); (3) not sensitized and no reaction since age 4 years (n = 447), no parental report of food avoidance, and parent-reported ingestion history (eaten >1 time since age 4 years; n = 2570). **C,** *Definite allergy* is defined as any of the following: (1) positive OFC result and IgE sensitized at age 6 years (n = 11); (2) history of objective reaction in the past 12 months consistent

TABLE E1. OFC protocol for tree nut challenges in the HealthNuts study

One level, 5-mL teaspoon	Average total weight in 1 level teaspoon (g)	Average total mg of nut protein in 1 level teaspoon
Almond meal	4.0	1200
Hazelnut meal	4.0	600
Crushed walnut	2-2.5	550
Crushed cashew	2.5-3.0	700
Macadamia nut	3.0	300
Pecan	2.5	250
Pistachio	3.0	600

with HealthNuts OFC stopping criteria after definite exposure to the food of interest and evidence of IgE sensitization at age 6 years (n = 6); or (3) positive OFC result at age 4 years and SPT response of 8 mm or greater at 6 years of age (n = 4). *Probable allergy* is defined as any of the following: (1) SPT response of 8 mm or greater but no age 4 years OFC or recent reaction history and no known tolerance (n = 23) or (2) SPT response of 3 to 7 mm at age 6 years and one of (A) positive OFC result at age 4 years, (B) history of objective reaction more than 12 months ago consistent with HealthNuts OFC stopping criteria after definite exposure to the food of interest, or (C) parental report of food avoidance because of allergy (n = 0). *Tolerant* is defined as any of the following: (1) negative OFC result (n = 23); (2) SPT response of 3 to 7 mm and parent-reported ingestion history (eaten >1 time since age 4 years; n = 30); (3) not sensitized and no reaction since age 4 years (n = 220); and (4) no parental report of food avoidance and parent-reported ingestion history (eaten >1 time since age 4 years; n = 2854).

TABLE E2. Summary of SPT, OFC, and allergy outcomes for additional tree nuts

Tree nut type (no. of SPTs performed)	Not Sensitized (<3 mm)	Sensitized (≥3 mm)		
		3-7 mm and OFC	3-7 mm, no OFC	≥8 mm
Brazil nut (n = 90)	70	0	15 (15 = missing)	5 (5 = probable allergy)
Macadamia nut (n = 101)	75	0	11 (3 = allergic, recent reaction; 8 = missing)	15 (14 = probable allergy; 1 = tolerant)
Pecan (n = 107)	80	0	19 (1 = allergic, recent reaction; 1 = tolerant; 17 = missing)	6 (6 = probable allergy)
Pistachio (n = 116)	43	5 (3 = positive; 1 = negative; 1 = inconclusive)	19 (6 = tolerant; 13 = missing)	49 (4 = tolerant; 45 = probable allergy)
Walnut (n = 111)	66	8 (5 = positive; 2 = negative; 1 = inconclusive)	17 (3 = tolerant; 14 = missing)	20 (3 = allergic at age 4 y; 17 = probable allergy)

Definite allergy is defined as any of the following: (1) positive OFC result and IgE sensitized at age 6 years; (2) history of objective reaction in the past 12 months consistent with HealthNuts OFC stopping criteria after definite exposure to the food of interest and evidence of IgE sensitization at 6 years; or (3) positive OFC result at age 4 years and SPT response at 8 mm or greater at 6 years of age. *Probable allergy* is defined as any of the following: (1) SPT response of 8 mm or greater but no age 4 years OFC or recent reaction history and no known tolerance or (2) SPT response of 3 to 7 mm at age 6 years and 1 of (A) positive OFC result at age 4 years, (B) history of objective reaction more than 12 months ago consistent with HealthNuts OFC stopping criteria after definite exposure to the food of interest, or (C) parental report of food avoidance because of allergy. *Tolerant* is defined as any of the following: (1) negative OFC result (n = 23); (2) SPT response of 3 to 7 mm and parent-reported ingestion history (eaten >1 time since age 4 years); (3) not sensitized and no reaction since age 4 years; or (4) no parental report of food avoidance and parent-reported ingestion history (eaten >1 time since age 4). *Missing* is defined as sensitized, no OFC, and unknown ingestion history of specified tree nut.

TABLE E3. Sensitization and allergy to almond, cashew, and hazelnut at age 6 years by using different definitions

	Any (almond, cashew, and hazelnut)				Almond				Cashew				Hazelnut			
	Sensitization ≥3 mm), % (95% CI)	Prob* (n = 69), % (95% CI)	Def† (n = 92), % (95% CI)	Overall‡ (n = 139), % (95% CI)	Sens (≥3 mm), % (95% CI)	Prob* (n = 10), % (95% CI)	Def† (n = 7), % (95% CI)	Overall (n = 17), % (95% CI)	Sens (≥3 mm), % (95% CI)	Prob* (n = 51), % (95% CI)	Def† (n = 70), % (95% CI)	Overall (n = 121), % (95% CI)	Sens (≥3 mm), % (95% CI)	Prob* (n = 23), % (95% CI)	Def† (n = 21), % (95% CI)	Overall (n = 43), % (95% CI)
Assessment group§ (observed prevalence [n = 3232])	7.3 (6.4-8.3)	2.7 (1.7-2.7)	2.3 (1.8-2.9)	4.3 (3.8-5.2)	2.8 (2.2-3.4)	0.3 (0.1-0.6)	0.2 (0.1-0.4)	0.5 (0.3-0.8)	5.0 (4.2-5.8)	1.6 (1.2-2.1)	2.2 (1.7-2.8)	3.8 (3.2-4.6)	3.3 (2.8-4.1)	0.7 (0.4-1.0)	0.7 (0.4-1.0)	1.3 (1.0-1.8)
Assessment group‖ (weighted prevalence [n = 3232])	5.8 (5-6.7)			3.3 (2.8-4.0)	2.2 (1.8-2.9)			0.3 (0.1-0.5)	3.9 (3.3-4.6)			2.7 (2.2-3.3)	2.5 (2.0-3.1)			0.9 (0.7-1.3)
Whole cohort¶ (n = 5276)		1.3 (1.0-1.7)	1.4 (1.1-1.8)	2.6 (2.3-3.2)		0.2 (0.1-0.3)	0.1 (0.04-0.2)	0.3 (0.2-0.5)		1.0 (0.7-1.3)	1.4 (1.1-1.7)	2.4 (2.0-2.8)		0.4 (0.3-0.7)	0.5 (0.3-0.7)	0.9 (0.7-1.2)

**Probable food allergy* is defined as any of the following: (1) SPT response of 8 mm or greater but no age 4 years OFC or recent reaction history and no known tolerance; (2) SPT responses of 3 to 7 mm at age 6 years and positive OFC result at age 4 years, (3) evidence of IgE sensitization at age 6 years with a history of objective reaction consistent with HealthNuts OFC stopping criteria after definite exposure to the food of interest and evidence of IgE sensitization, or (4) SPT response of 3 to 7 mm and parental report of food avoidance because of allergy.

†*Definite food allergy* is defined as any of the following: (1) positive OFC result and IgE sensitized at age 6 years; (2) history of recent objective reaction in the past 12 months consistent with HealthNuts OFC stopping criteria after definite exposure to the food of interest and evidence of IgE sensitization at age 6 years; or (3) positive OFC result at age 4 years and SPT response of 8 mm or greater at age 6 years.

‡*Overall any tree nut allergy*: Numbers do not add to the total of probable and definite food allergy because some participants had multiple nut allergies.

§*Assessment group (observed prevalence)*: Children who had an allergy/health assessment completed at age 6 years, which included SPTs to 8 foods (milk, egg, peanut, wheat, sesame, cashew, hazelnut, and almond). Those with negative SPT responses to all 3 tree nuts were deemed tree nut tolerant.

‖*Assessment group (weighted prevalence)*: Children who had an allergy/health assessment completed at age 6 years, which included SPTs to 8 foods (milk, egg, peanut, wheat, sesame, cashew, hazelnut, and almond). Those with negative SPT responses to all 3 tree nuts were deemed tree nut tolerant. Prevalence estimates were calculated by weighting the proportion of the participants who had a full clinic assessment at age 6 years by using sampling weights equal to the inverse probability of the family participating in the study at age 1 year.

¶*Whole cohort*: All children who participated in the HealthNuts study at age 1 year. Those who did not participate in follow-up at age 6 years or had unknown tree nut exposure at age 6 years were assumed to be tree nut tolerant (n = 1975).

7. IMPROVING THE DIAGNOSIS OF CASHEW ALLERGY

7.1 Introduction

This chapter is presented in the form of a manuscript that has been submitted for publication to the Journal of Allergy and Clinical Immunology and Allergy in October 2018.

As for other forms of food allergy, tree nut allergy diagnosis is primarily via SPT and sIgE together with OFC. The application of 95%PPVs can assist clinicians with the decision of when an OFC may be warranted, however published 95% PPV data for tree nuts remains limited.

Previous studies as part of this PhD have found cashew to be the most common individual tree nut allergy and the most common trigger of adverse reactions in Australia highlighting a priority need for improved diagnostic tools for cashew allergy.

This final manuscript as part of this PhD addresses the following research questions:

1. What are the SPT wheal sizes that correlate with a 95% PPV of a positive oral food challenge for cashew?
2. Do these thresholds differ when stratified by allergy clinic or general population cohorts?
3. Do these thresholds differ when stratified by known risk factors for food allergy?

7.2 The Manuscript

SPT predictive values for the outcome of cashew challenges in children

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The SchoolNuts investigators who are not individually named as authors are Susan Sawyer, George Patton, Jo Douglass, and Peter Vuillermin.

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Abstract

Background: Cashew is a common cause of tree nut allergy in children. To date there have been few studies of diagnostic tests for cashew allergy, and positive predictive values (PPVs) for cashew as well as other tree nuts are largely extrapolated from studies of peanut allergy. How relevant these cut-offs are for cashew has not been formally explored.

Objective: We aimed to establish skin prick test (SPT) wheal sizes that correlated to 95% PPV for a positive food challenge for cashew.

Methods: We included all cashew oral food challenges (OFC) conducted as part of the HealthNuts (n=108, age 4-6 years) and SchoolNuts (n=37, age 10-14 years) studies, both recruited from the community (Population cohort). A second cohort of all cashew OFCs conducted at the Royal Children's Hospital (RCH) allergy centre (n=343) (2011-2016) and a private allergy clinic based at RCH (n=43) was included via electronic medical record review (Clinic cohort). 95% PPV for cashew SPT was calculated for both cohorts.

Results: Among the population cohort (n=145), 62% of cashew OFC were positive compared to 20% of the clinic cohort (n=386)**.** The SPT threshold for 95% PPV derived from the population cohort was 10mm (95%CI 7.5-12.0). For the clinic cohort the 95% PPV was 14mm (95%CI 9.5-unknown). A SPT wheal size of 8mm had a PPV of 89 (95%CI 79-95) in the population cohort and 62 (95%CI 45-78) in the clinic cohort.

Conclusion: A higher SPT wheal size may be more appropriate than the commonly used 8mm cut-off to guide clinical decisions around when to perform OFC for cashew.

Word count = 258

Key words: food allergy, tree nut allergy, cashew allergy, population, predictive value of tests, skin prick test

Abbreviations:
sIgE: serum immunoglobulin E
OFC: oral food challenge
LR: likelihood ratio
NPV: negative predictive value
PPV: positive predictive value
ROC: receiver operating characteristic
AUC: area under the curve
SPT: skin prick test

Introduction

Tree nut allergies, like peanut allergy, are thought to be increasing in prevalence, affecting up to 3% of Australian children[1]. Cashew has been reported as the most common tree nut allergen and the most common cause of adverse food reactions and anaphylaxis [2-4]. It has also been reported that reactions to cashew can be more severe than those to peanut [5-8]. Despite these reports, cashew allergy remains understudied.

Diagnosis of IgE-mediated food allergy is reliant on a detailed medical history and physical examination, in conjunction with diagnostic testing of immunoglobulin E (IgE) levels either via serum specific IgE measurements (sIgE) or skin prick testing (SPT)[9]. Diagnosis of cashew allergy, like other tree nuts, can be problematic with allergy screening tests often performed for tree nuts for those with allergy to other foods and no history of prior ingestion or reaction to cashew. We have previously reported that up to half of those with egg and peanut allergy can be sensitised on SPT to tree nuts as early as one year of age[1] . The diagnostic gold standard is to perform an oral food challenge (OFC), however this is time and labour intensive, limited in availability in many tertiary allergy services and exposes the child to the risk of severe reactions [10]. This can result in patients being advised to avoid multiple tree nuts based on sensitisation alone, which, given recently published evidence of the protective effect of early allergen introduction, may increase their risk of developing tree nut allergy [11].

SPT and sIgE thresholds above which the individual has a 95% probability of being clinically allergic (95% positive predictive value (PPV)) have been used as a tool for clinicians to determine when an OFC should be performed. In Australia, SPTs are performed in preference to sIgE in most paediatric allergy clinics. PPVs are dependent on the underlying prevalence of disease (in other words, the pre-test probability of food allergy), which is likely to depend on factors such as age, SPT method and the population in which the allergy testing is being performed [12, 13]. Clinic-based PPV studies often do not report the reason for challenge, however in our experience most OFC in the clinical setting are undertaken to determine resolution of allergy (tolerance) rather than a diagnostic challenge. Those at higher risk of reaction, such as those with high SPT wheal sizes are not offered an OFC, which impacts the pre-test probability of food allergy.

PPVs for SPT have now been widely published for peanut, egg, milk and sesame in both clinical and population cohorts [14-26]. There is limited data on tree nut PPVs and no data derived from

population cohorts[16, 27]. With the possibility of increasing prevalence and severity of cashew allergy there is an urgent need for more accurate, timely and cost effective diagnostic methods.

We aimed to establish the SPT wheal size that correlated with 95% positive predictive value to a positive oral food challenge for cashew in a population-based cohort of children and a clinic-based cohort of children. We also aimed to explore if these thresholds differ when stratified by known risk factors for cashew allergy (co-existing peanut or other food allergy, co-existing atopy, previous reaction history, age and sex).

Methods

Study Populations:

The population cohort of this study comprised of participants who underwent cashew OFC as part of the HealthNuts and SchoolNuts studies. The HealthNuts and SchoolNuts studies are two large population-based allergy studies done at the Murdoch Children's Research Institute. Both studies recruited participants from the community. Cashew OFCs were performed at 4-year-old and 6-year-old follow-up in the HealthNuts study, whereas the SchoolNuts study assessed students aged 10-14 years. The study populations are outlined in further detail below.

The HealthNuts study is a population-representative longitudinal study of 5276 children recruited at age 1 and followed up to age 6 years. The study methods have been described in detail previously [28-30]. To summarise, between 2007 and 2011, 5276 infants aged between 11-15 months were recruited from immunisation clinics around Melbourne, Australia (74% participation). At recruitment all infants underwent skin prick testing (SPT) to four common allergens (peanut, egg, sesame and milk), those with detectable SPT responses were invited to the Royal Children's Hospital Melbourne for a formal OFC (83% of those eligible attended).

At age 4 years all participants were followed up via questionnaire (83% participation) and those who reported a new food allergy reaction, and those who had any food allergy at age 1 year, were invited for clinic assessment that included SPT to a predetermined panel of 8 foods (milk, egg, peanut, wheat, sesame, cashew, almond and hazelnut). All those with a SPT>=1mm were offered an OFC.

At age 6 years the entire cohort (n=5276) were invited to participate in questionnaire and SPT assessment. Questionaries were mailed to all participants capturing demographic details, history of food allergy and new food reactions, common allergen exposure information, history of asthma/wheeze and eczema. All participants were invited for an allergy/health assessment that included SPT to the same predetermined panel of 8 foods as age 4 years (60.5% participation). All those with a positive SPT (>=1mm) were offered a cashew OFC except those that had a cashew positive OFC at age 4 years and a cashew SPT >=8mm at 6 years or a recent history of IgE-mediated reaction.

The SchoolNuts study is a cross-sectional population-based study that aimed to determine the population prevalence of challenge-proven food allergy in early adolescence. The study methods have been described in detail previously [2, 31]. Briefly, recruitment was via stratified random population-based sampling of schools in Melbourne (n=9663) from 2011-2014. Questionnaires with phone follow up were used to identify students with potential IgE-mediated food allergy. Students with potential IgE mediated food allergy were invited to attend a clinic assessment for allergy testing (SPT) to a panel of 15 food allergens (egg white, cow's milk, soy, peanut, cashew, almond, hazelnut, walnut, pistachio, macadamia, pecan, brazil nut, pine nut, sesame, shellfish) and a panel of environmental allergens.

Students were eligible for OFC if they had a positive SPT result (≥3mm) to a food that they had a history of reaction to and were currently avoiding, or that they had never eaten. OFC were not performed if participants met any one of the following criteria: 1. were sensitised (≥3mm) and had a past history of a severe reaction requiring multiple doses of adrenaline, 2. Reported an episode of anaphylaxis when older than 10 years of age, 3. reported a food reaction in the past 12 months consistent with an IgE-mediated food allergy reaction, or 4. were highly sensitised (≥8mm) and reported a past history of reaction consistent with IgE-mediated food allergy.

The clinic cohort of this study comprised of patients who had undergone a cashew OFC as part of the allergy clinic at the Royal Children's Hospital (RCH) between 2011 and 2016 and a private allergy clinic-based at RCH, the Melbourne Allergy and Children's Centre (MACCS), that opened in 2015 and had an OFC database established from 2016. We conducted a retrospective analysis of all sequential open cashew OFCs undertaken at both sites. Patients undergoing cashew OFC were identified from the electronic medical records databases at both the RCH and MACCS. Cashew SPT wheal size, co-existing food allergy, current and resolved comorbid allergic disease

and food and environmental allergen sensitisation were extracted via chart review. Those with their most recent cashew SPT conducted more than 3 months prior to OFC were excluded.

Skin prick testing:

All SPT performed at RCH, MACCS and the HealthNuts study were performed on the child's back, while the SchoolNuts study utilised the volar aspect of the student's forearm. Wheal size was measured after 15 minutes and calculated by subtracting the negative control from the average of the longest diameter and the diameter perpendicular to it. SPTs for MACCS and RCH were performed with Quintip device (Stallergenes, Antony, France) and the HealthNuts and SchoolNuts studies were performed with single-tine lancets (Stallergenes, Antony, France) using cashew extract along with a positive and negative saline control. Extracts for the HealthNuts and SchoolNuts studies were ALK-Abello SA, Madrid, Spain and RCH and MACCS Hollister-Stier, Stallergenes, Antony, France. All SPT were performed within 3 months prior to cashew OFC.

Oral food challenges:

Criteria for undergoing cashew oral food challenges

HealthNuts participants with a cashew SPT ≥ 1mm or a parent-reported reaction consistent with an IgE-mediated allergy were invited for a clinic appointment with a specialist allergy nurse and OFC. The low SPT cut-off for sensitisation was applied in the HealthNuts study to ensure that all potential cases of food allergy were detected. Cashew OFC were offered at both the 4-year-old and 6-year-old follow up.

SchoolNuts participants with a parent-reported history of an adverse food reaction consistent with IgE-mediated food allergy were invited to participate in an allergy assessment which included cashew SPT. Those with a cashew SPT ≥3mm were invited for cashew OFC.

Cashew OFC protocols

OFC dosage protocols for all sites were consistent with those of the Australian Society of Clinical Immunology and Allergy (ASCIA) using graded, incremental doses administered at 15- to 20-minute intervals with a top dose of 2 teaspoons of crushed cashew. OFC protocols for the HealthNuts and SchoolNuts studies have been previously described[2, 32]. An OFC was deemed positive if it met at least one of the following predefined criteria: (1) three or more concurrent non-contact urticaria lasting at least 5 minutes; (2) severe persistent vomiting; (3) peri-oral or peri-orbital angioedema; or (4) anaphylaxis (evidence of circulatory or respiratory involvement) within 2 hours of the last challenge dose in the presence of IgE sensitisation. For the SchoolNuts study, additional criteria based on persistent subjective symptoms in the upper airways or the

gastrointestinal tract were included due to the older age of participants. In the absence of objective signs, if subjective symptoms (itchy mouth or throat, abdominal pain or nausea, tightness in throat, difficulty talking or difficulty breathing) continued up to the timing of the next dose, the previous dose was repeated. If the above symptoms persisted for a total of more than 40 minutes or reoccurred on 2 doses, the challenge was considered positive.
OFCs were deemed negative at all sites if a patient or participant tolerated a top dose of 2 teaspoons of crushed cashew nut on the day of the OFC and did not report any positive reactions during continued daily intake of 2 teaspoons at home in the week after the OFC.

RCH and MACCS cashew OFCs were performed in a clinical allergy setting with the recommendation to undertake an OFC based on the clinical expertise of the attending allergist. In this setting OFCs are not routinely performed if there is a high likelihood of reaction such as those with high-level sensitisation or a recent history of reaction. Therefore, OFCs are largely performed to determine tolerance rather than diagnose food allergy. Occasionally, an OFC may be performed despite a high positive SPT if there is a recent history of tolerance to a partial ingestion of cashew. All RCH and MACCS OFCs had symptoms retrospectively reassessed and those not meeting OFC stopping criteria as outlined by PRACTALL guidelines were excluded (n=2) [33].

Statistical Methods:

Continuously-valued variables are summarised using means (and standard deviations) or medians (and range), with frequencies reported as percentages with 95% confidence intervals based on the binomial distribution. A two-sample comparison of the prevalence of positive OFC within the clinic cohorts showed little evidence these samples were drawn from populations with different prevalence of cashew allergy ($p = 0.24$) and similarly for the population-based cohorts ($p = 0.24$).
There was no evidence against the null hypothesis of a linear relationship between the SPT wheal size and the log-odds of a positive OFC based on data from any of the cohorts, so we assumed linear association throughout our analyses.

In light of these results, we combined data for the two clinic cohorts RCH and MACCS to form a clinic cohort and for the two population-based cohorts HealthNuts and SchoolNuts to form a population cohort for all analyses. All analyses were conducted for the population and clinic cohorts separately. The capacity of the cashew skin prick test to diagnose OFC-confirmed cashew allergy was assessed using receiver operating characteristic (ROC) curves and the area

under the curve (AUC) was used to quantify the accuracy of the test. Logistic regression was used to model the association between the SPT wheal size in millimetres and the risk of cashew allergy. The assumption that the logarithm of the odds of food allergy was linearly related to the SPT wheal size was assessed using the likelihood ratio test to compare the linear model with a more general "saturated" model that made no assumption about the shape of the relationship between SPT wheal size and the risk of food allergy. A fitted probability of food allergy was produced for each study participant given their SPT wheal size and used to replace the observed binary outcome in the standard formula for the PPV that is, a modelled PPV for each level of SPT wheal size was produced by taking the average of the fitted probability of cashew allergy for all infants with an SPT wheal size of greater than the given level. This method produces a smooth non-decreasing curve for the PPV across the range of SPT wheal sizes therefore overcoming fluctuations (sampling variation) in the observed proportion of infants with cashew allergy for increasing SPT. To quantify the precision of estimation of the PPVs, we used bootstrapping, a method of deriving SEs and CIs from repeated samples drawn with replacement from the original dataset. One hundred bootstrap replications were used to determine the variability of parameter estimates and to calculate 95% CIs for the thresholds with 95% PPVs to food allergy.

The analysis was stratified on known risk factors for food allergy/positive tree nut challenge: sex, co-existing peanut allergy, co-existing tree nut allergies, co-existing non-nut allergies, other allergic disease and previous reaction history. All analyses were done using Stata 15.0 (release 15.0, StatCorp, College Station, Texas).

Ethics:

Approval to conduct the HealthNuts study was obtained from the Victorian State Government Office for Children (reference no. CDF/07/492), the Victorian State Government Department of Human Services (reference no. 10/07) and the RCH Human Research Ethics Committee (reference no. 27047). For the SchoolNuts study, ethics approval was obtained from the RCH Human Research Ethics Committee (HREC number 31079), the Department of Education and Early Childhood and the Catholic Education Office. All parents provided written informed consent.

Ethics approval for the use of de-identified clinic data from RCH and MACCS was obtained from the RCH Human Research Ethics Committee (HREC number 37076A).

Results

Study Populations:

Demographic and clinical characteristics stratified by cashew OFC cohort are outlined in Table 1. A total of 386 cashew OFC were performed in a clinical setting (RCH n=343, MACCS n=43) and 145 cashew OFC were performed as part of the population-based studies (HealthNuts n=108, SchoolNuts n=37). Males represented 61% of the clinic cohort and 57.9% of the population cohort. The median age at challenge was 8 years for the clinic cohort and 6 years for the population cohort. Co-morbid rates of eczema were comparable between cohorts [54.7% (211/386) clinic cohort, 55.1% (80/145) population cohort], whilst rates of asthma and rhinitis were higher among the clinic cohort [clinic cohort asthma 58.8% (n=227), population cohort asthma 29.6% (n=43); clinic cohort rhinitis 47.7% (n=184) and population cohort 18.8% (n=23)]. Among the clinic cohort, 63.5% (n=245) had a co-existing food allergy (peanut n=194, other tree nut n=39, egg n=62, other= 62) compared to 51% (n=74) of those among the population cohort (peanut n=33, other tree nut n=12, egg n=13, other n= 14). Age stratified demographic and clinical characteristics for the clinic cohort are outlined in Supplementary Table 1.

Results of cashew OFC:

Among the clinic cohort, 19.9% (n=77) of cashew OFC were positive compared to 62.1% (n=90) of the population cohort challenges. The most common reason for cashew OFC among both cohorts was cashew sensitisation without a history of cashew ingestion, 46.6% of those in the clinic cohort and 67.7% of those among the population cohort. Among the population cohort, 91% (n=61) of those with an SPT≥ 8mm and no history of cashew exposure had a positive cashew OFC compared to 59% (n=22) of those among the clinic cohort.

Characteristics of the positive cashew OFCs

Characteristics of positive cashew challenges stratified by cohort are outlined in Table 2. Among those in the clinic cohort with a positive cashew challenge (n=77) the most common symptoms were skin (75%), gastrointestinal (48%) and oropharyngeal (38%). Among those in the population cohort with a positive cashew challenge (n=90) the most common symptoms were angioedema (61%), skin (60%) and oropharyngeal (50%). There were marked differences in reported treatments between the clinic and population cohorts. Among those with a positive cashew OFC in the population cohort (n=90), 77% required no treatment, 22% antihistamine and 5.6% salbutamol. Among those with a positive cashew OFC in the clinic cohort (n=77), 19.5% required no treatment, 78% antihistamine, 3.4% salbutamol and 1.3% oral steroid.

Among the 168 positive cashew challenges in the population and clinic cohorts, nine children required adrenalin (5.3%). Table 3 summarises the details of the nine patients requiring adrenalin. Five had no history of cashew ingestion and three had a previous history of cashew reaction (one anaphylaxis) and cashew SPT wheal sizes ranged from 1-18mm. Five had co-existing food allergy (milk 2, sesame 2 and another tree nut 1).

The majority of reactions among the population cohort occurred with a crumb (52%, n=47) whereas reaction doses in the clinic population tended to be higher with 29/77 (38%) occurring at the top dose (2 teaspoons) (Table 2).

Diagnostic capacity of cashew SPT for challenge confirmed cashew allergy:

There were marked differences in the prevalence of cashew allergy between the population and clinic-based cohorts 62.1% and 19.5% respectively (see Table 1), which we believe is due to differences in the selection of participants for cashew challenge between the 2 cohorts. Patients in the allergy services from which the clinic cohort is drawn are *less* likely to have undergone an OFC if the attending clinician determines that they are *more* likely to have a positive result, such as patients with high level sensitisation (>8mm SPT). This is not the case for the population-based cohorts, where OFC were offered to all participants with a detectable SPT (wheal size ≥1mm except those with a recent history of a reaction consistent with IgE-mediated food allergy (Supplementary Table 2). This is further supported by the difference in median SPT between the 2 cohorts [clinic cohort=3mm (IQR 1-5), population cohort=7.5mm (IQR 4-11)]. The SPT threshold with 95% PPV for cashew allergy also varied between the population and clinic-based cohorts, 10mm or greater (7.5-12.0) and 14mm or greater (95%CI 9.5-unknown) respectively. AUC was 0.89 (95% CI 0.83-0.95) for the population cohort and 0.81 (0.76-0.87) for the clinic cohort. SPT thresholds with 95% PPV for cashew allergy, along with sensitivity, specificity, NPV, positive and negative LR at the reported thresholds are presented in Table 4.

When stratified by risk factors for cashew allergy, 95% PPVs could not be generated for the clinic cohort. For the population cohort some differences were noted but with overlapping confidence intervals there is little evidence to support differences in 95% PPVs between the groups at the population level. (Supplementary Table 4). There was no evidence of differences in population-level 95% PPVs based on analyses after stratification on other known risk factors for food allergy or age (data not shown).

The commonly applied SPT thresholds of less than 3mm and 8mm and above are presented in Table 5. The 3mm threshold performed as expected with high sensitivity and low specificity for the population and clinic-based cohorts.

Discussion:

To our knowledge this is the largest series of oral food challenges for cashew allergy reported and the first study to report 95% PPVs for cashew SPT. We found the SPT threshold for 95% PPV among a clinic cohort of 14mm and a population cohort of 10mm with marked differences in cashew allergy prevalence between the clinic and population cohorts.

The strengths of this study are the large number of cashew OFCs and the development of SPT thresholds with 95% PPVs for cashew allergy using data contributed from population and allergy clinic-based samples of children in the same city. The population-based cohorts provide cashew OFC outcomes based on pre-determined stopping criteria irrespective of SPT wheal size.

There are also a number of limitations. Similar to other studies based on data from allergy clinics, the results of our analyses of data from the clinic-based cohorts are affected by two types of selection bias since patients are likely to have (1) more severe allergic disease when compared to individuals from population cohorts of children; and (2) been offered OFC only if the allergist deemed them to be at low risk of reacting. As a result, the clinical cohort has a limited number of OFCs represented with high-level sensitisation (>8mm), a lower median SPT and the majority of reactions were to the top OFC dose. The clinic cohort data are also likely to have suffered from a type of information bias, since details were obtained from a retrospective record review and OFC outcomes were not standardised. All challenge outcomes were, however, reviewed and those that did not meet the PRACTALL criteria[33] for a positive oral food challenge were excluded from analysis. Both the population and clinic cohorts have a limited number of cashew OFC performed in children <2 years of age which is the age that children typically present with food allergy and when 95% PPV estimates for cashew SPT would be most useful to guide cashew introduction recommendations.

Comparison of our thresholds for cashew SPT response to other studies is difficult due to the limited number of studies to date. One study reported that SPT wheal diameters ≥8mm predicted a positive food challenge with >95% accuracy individually for cashew, hazelnut, walnut, and sesame. This study was, however, from a highly selected clinic population and

included lower numbers of cashew OFC (n=89) [16]. A more recent study that retrospectively reported outcomes of tree nut challenges from the University of Michigan Allergy clinic included 28 cashew challenges and fitted a single predictive model that combined cashew and pistachio OFCs to generate a 50% NPV of 6.5mm.

If the sensitivity and specificity of SPT thresholds for OFC allergy are assumed not to vary between populations, then PPVs and NPVs will be dependent on the underlying prevalence of food allergy. It has been previously argued that PPVs for sIgE derived from clinic populations cannot be meaningfully applied to general populations, although this question has not yet been addressed using relevant data on SPT wheal size [13]. Our data demonstrates a similar variation of PPVs based on different populations and highlights that PPVs based on clinic cohorts are more likely to include those with lower prevalence of food allergy due, most likely, to selection bias related to physician decisions around who is offered an oral food challenge, and therefore generate artificially higher PPV thresholds. Based on our results we propose that a SPT wheal size of 10mm may be more appropriate than the commonly used 8mm cut-off to guide clinical decisions around when to perform OFC for cashew.

It has been proposed that likelihood ratios, which compare the probability of a positive SPT result in patients with and without food allergy, should be the tool of choice to determine the probability of allergic disease as they have the potential to be independent of disease prevalence [34]. The calculation and appropriate application of likelihood ratios does, however, require several steps, which may explain why this procedure has received limited use in clinical practice. In the present study, we have included both positive and negative likelihood ratios to assist clinicians with applying our data to their own settings.

Conclusion

Within a population-based cohort we have established a 95% PPV for cashew SPT of 10mm. It is known that 95% PPVs can vary depending on the population they are generated from and this study has demonstrated considerable variability between clinic- and population-based cohorts for the 95% PPV for cashew SPT. To improve tree nut allergy diagnosis and management, further work is required utilising data based on OFC confirmed outcomes with OFC offered to all sensitised individuals irrespective of wheal size to generate 95% PPVs for the additional tree nuts.

Figure Legend

Fig 1a. ROC curve for cashew SPT wheal size, population cohort.

Fig 1b. ROC curve for cashew SPT wheal size, clinic cohort.

Table Legend

Table 1: Demographic and clinical characteristics, stratified by cashew OFC cohort

Table 2: Characteristics of positive cashew oral food challenges, stratified by cohort

Table3: Details of the participants with a positive OFC that required adrenalin (n=9)

Table 4: Diagnostic capacity of SPTs to challenge-confirmed cashew allergy

Table 5: Diagnostic capacity of commonly applied SPT thresholds (<3mm and > 8mm) to challenge-confirmed cashew allergy

Table 1: Demographic and clinical characteristics, stratified by cashew OFC cohort

	Clinic Cohort n=386		Population Cohort n=145	
	RCH n=343	**MACCS n=43**	**HealthNuts n=108**	**SchoolNuts n=37**
Gender, male n (%)	210 (61.2)	26 (60.5)	62 (57.4)	22 (59.5)
Mean Age at OFC, yrs (SD)	8.0 (4.4)	7.2 (4.9)	5.2 (1.1)	12.9 (1.1)
Challenge Outcome, n (%)				
Positive	65 (18.9)	12 (27.9)	68 (62.9)	22 (59.4)
Negative	260 (75.8)	31 (72.1)	39 (36.1)	12 (32.4)
Equivocal	14 (4.1)	0	1 (0.9)	3 (5.4)
Missing	4 (1.2)	0	0	0
Median SPT, mm (interquartile range)				
Overall SPT	3(1-5)	3(1-4)	8 (4-12)	6 (2-9)
SPT, positive challenge	6 (4-8)	6 (4-8)	11 (8-14)	8 (6-10)
SPT, negative challenge	3 (0-4)	3 (0-4)	4 (0-6)	1 (0-4)
Reason for Cashew OFC, n (%)				
Not cashew sens (<3mm) or allergic but allergy to other nut/s	35 (10.2)	3 (7.0)	1 (0.9)	5 (13.5)
Not cashew sens (<3mm) or allergic, other non nut allergies	24 (7.0)	5 (11.6)	3 (2.8)	0
Not cashew sens (<3mm) or allergic, no other food allergies	25 (7.3)	1 (2.3)	14 * (13.0)	0
Sensitised 3-8mm , never eaten cashew	144 (42.1)	16 (37.2)	29 (26.9)	6 (16.2)
Sensitised >8mm, never eaten cashew	20 (5.9)	2 (4.7)	50 (46.3)	11 (29.7)
Previous cashew reaction	70 (20.5)	11 (25.6)	9 (8.3)	14 (37.8)
Reaction to an unknown trigger	14 (4.1)	5 (11.7)	0	0
Unable to determine OFC reason	10 (2.9)	0	2 (1.9)	1 (2.7)
Food Allergy Details, n (%)				
No other food allergies	124 (29.0)	15 (34.9)	58 (53.7)	13 (35.1)
Co-existing food allergy	220 (64.0)	25 (58.1)	50 (46.3)	24 (64.9)
Peanut allergy	76 (22.2)	8 (18.8)	23 (21.3)	11 (29.7)
Other tree nut €	36 (10.5)	3 (7.0)	2 (1.9)	10 (27.0)
Egg allergy	54 (15.7)	8 (18.6)	11 (10.2)	2 (5.4)
Other allergies β	53 (15.5)	9 (20.9)	13 (12.0)	1 (2.7)
Food sensitisation details, n (%)				
Peanut 3-8mm	5 (4.3)	1 (4.3)	3 (6.8)	1 (4.2)
Peanut>8mm	5 (17.2)	1(14.3)	5 (19.2)	1 (20.0)
Any tree nut 3-8mm #	80 (68.4)	18 (78.3)	31 (70.5)	11 (45.9)
Any tree nut >8mm £	18 (62.1)	6 (85.7)	19 (10.5)	3 (60.0)
Egg 3-8mm	4 (3.4)	0	3 (60.0)	1 (4.2)
Egg >8mm	0	0	0	0
Sesame 3-8mm	8 (6.8)	2 (8.7)	3 (6.8)	3 (12.5)
Sesame >8mm	5 (17.2)	0	1 (3.9)	0
Milk 3-8mm	0	0	1 (3.9)	2
Milk >8mm	0	0	0	0
Shellfish 3-8mm	1 (3.4)	0	3 (6.8)	0
Shellfish >8mm	0	0	0	1 (20.0)
Allergic disease history, n (%) ¶				
Current eczema	194 (56.6)	17 (39.5)	60 (55.6)	20 (54.1)
Current asthma	197 (57.4)	30 (69.8)	29 (26.9)	14 (37.8)
Current rhinitis	157 (45.8)	27 (62.8)	16 (14.8)	7 (18.9)

Table 1 Legend

*HealthNuts study protocol was to offer all participants with SPT≥1mm an OFC to ensure all potential cases of food allergy were captured.

€ Tree nut allergy: defined as a positive OFC or history of reaction and sensitised (≥3mm). Does not include those with SPT≥8mm defined as probable allergy in the HealthNuts (n=19) and SchoolNuts studies (n=3) Individual tree nut allergy details -almond=2, hazelnut=8, macadamia=7, pecan=4, pistachio=14, walnut=29

β Other food allergies: milk=26, sesame=23, fish=11, shellfish=4, wheat=4, kiwifruit =9

#Tree nut sensitisation 3-8mm: almond=18, hazelnut=30, macadamia=11, pecan=16, pistachio=72, walnut=44

£ Tree nut sensitisation >8mm: almond=3, hazelnut=15, macadamia=1, pecan=5, pistachio=21, walnut=15

¶ Current eczema, current asthma, current rhinitis=parent-report of doctor diagnosed eczema, asthma, rhinitis.

Table 2: Characteristics of positive cashew oral food challenges, stratified by cohort

	Clinic Cohort (n=77)	Population Cohort (n=90)
Mean age at OFC , years (SD)	7.8 (4.3)	7.1 (3.5)
Median SPT, mm (interquartile range)	6.0 (4-8)	10.0 (7-13)
OFC Top Dose		
Crumb/Smear	0	47 (52.2)
1/16	3 (3.9)	9 (10.0)
1/8 tsp	1 (1.3)	11 (12.2)
¼ tsp	11 (14.3)	8 (8.9)
½ tsp	6 (7.8)	10 (11.1)
1 tsp	22 (28.6)	1 (1.1)
2 tsp	25 (32.5)	4 (4.4)
Missing	9 (11.7)	0
Symptoms		
Skin (*pruritus, hives, rash)*	58 (75.4)	54 (60.0)
Angioedema	16 (20.8)	55 (61.1)
Oropharyngeal *(sneezing, itchy mouth/throat, eye/nose rubbing, rhinorrhea)*	29 (37.7)	44 (48.9)
Respiratory *(Wheeze, cough, stridor)*	18 (23.4)€	12 (13.3)£
Gastrointestinal (*nausea, abdo pain, vomiting)*	37 (48.1)	32 (35.6)
Cardiovascular *(tachycardia, dizziness, BP drop)*	0	0
Other (*distress, decreased activity)*	3 (3.9)	2 (1.1)
Treatment		
Nil	15 (19.5)	67 (77.4)
Antihistamine	60 (77.9)	20 (22.2)
Adrenalin	8 (10.4)	1 (1.1)
Salbutamol	3 (3.9)	5 (5.6)
Steroid	1(1.3)	0
Other	0	0

€ Clinic cohort respiratory symptom details: 8= intermittent cough, 2=throat clearing, 4=wheeze, 4=persistent cough
£Population cohort respiratory symptom details: 9=intermittent cough, 2=throat clearing, 1=wheeze

Table3: Details of the participants with a positive OFC that required adrenalin (n=9)

	Cohort	Age at OFC (yrs)	Cashew SPT (mm)	OFC reason	Other co-existing food allergy	Other allergic disease	OFC top dose (g)
1	RCH	12	1	Previous IgE-mediated reaction	None	Eczema	10
2	RCH	3	2	Reaction to unknown trigger	None	Eczema	10
3	MACCS	10	4	Previous IgE-mediated reaction	None	Rhinitis	10
4	RCH	3	5	Previous anaphylaxis to cashew	None	Eczema	NA
5	RCH	8	5	Sensitised, never eaten	Sesame	Eczema	NA
6	RCH	2	6	Sensitised, never eaten	Other tree nut	Eczema	2.5
7	RCH	9	9	Sensitised, never eaten	Milk	Eczema	10
8	RCH	16	12	Sensitised, never eaten	Milk	Rhinitis	2.5
9	HealthNuts	7	17.5	Sensitised, never eaten	Sesame	Eczema	0.31

Table 4: Diagnostic capacity of SPTs to challenge-confirmed cashew allergy

	95% PPV mm (95% CI)	NPV % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)	AUC (95% CI)
Population Cohort (HealthNuts & SchoolNuts)	10 (7.5-12)	47.5 (37.3-57.8)	42.9 (32.5-53.7)	97.9 (88.9-99.9)	20.6 (2.9-145.2)	0.6 (0.5-0.7)	0.89 (0.83-0.95
Clinic Cohort (RCH & MACCS)	14 (9.5-unknown)	79.7 (75.1-83.7)	7.6 (2.8-15.8)	99.3 (97.5-99.9)	10.9 (2.2-53.1)	0.9 (0.87-0.99)	0.81 (0.76-0.87)

Table 5: Diagnostic capacity of commonly applied SPT thresholds (<3mm and > 8mm) to challenge-confirmed cashew allergy

	SPT Wheal size	PPV % (95% CI)	NPV % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)	AUC (95% CI)
Population Cohort	**3mm**	76.6 (67.6-84.1)	85.7 (67.3-96.0)	95.5 (88.9-98.8)	48.0 (33.7-62.6)	1.84 (1.40-2.41)	0.09 (0.03-0.25)	0.72 (0.64-0.79)
	8mm	88.7 (79.0-95.0)	61.8 (49.2-73.3)	70.8 (60.2-79.9)	84.0 (70.9-92.8)	4.42 (2.31-8.47)	0.35 (0.25-0.49)	0.77 (0.70-0.84)
Clinic Cohort	**3mm**	30.8 (24.9-37.3)	93.6 (88.1-97.0)	88.6 (79.5,94.7)	45.5 (39.6-51.4)	1.63 (1.42-1.85)	0.25 (0.13-0.47)	0.67 (0.63-0.72)
	8mm	62.2 (44.8-77.5)	83.0 (78.5-86.9)	29.1 (19.4-40.4)	95.1 (92.0-97.3)	6.0 (3.2-11.1)	0.75 (0.65-0.86	0.62 (0.57-0.67)

Fig 1a. ROC curve for cashew SPT wheal size, population cohort.

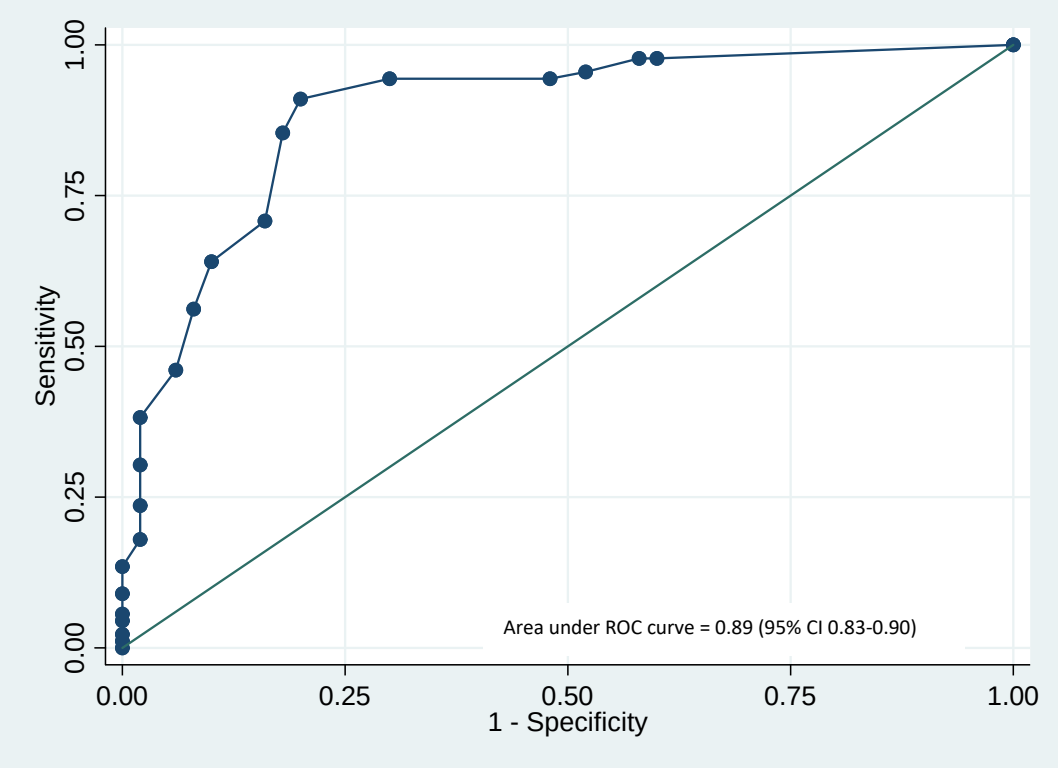
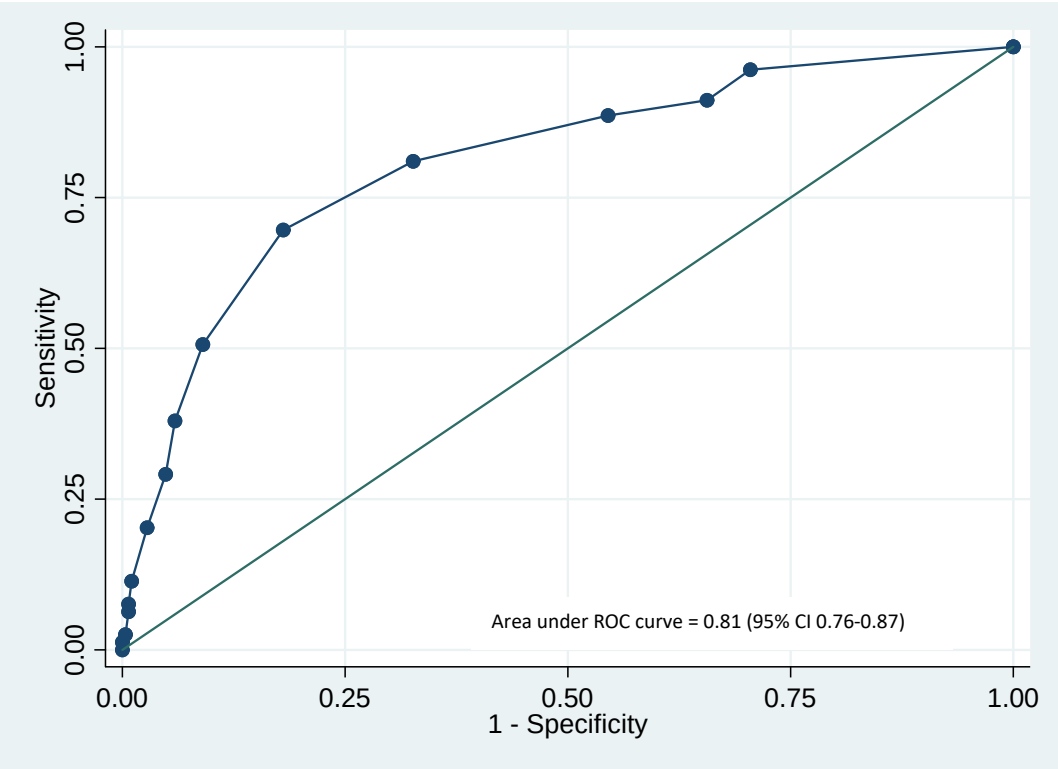


Fig 1b. ROC curve for cashew SPT wheal size, clinic cohort.



Supplementary Table 1. Demographic and clinical characteristics of the Clinic Cohort (RCH and MACCS), stratified by age

	0-2yrs n=50	3-9yrs n=304	10-14yrs n=137	15-18yrs n=40
Gender, male n (%)	36 (72.0)	107 (54.6)	65 (63.7)	28 (75.7)
Challenge Outcome				
Positive	11 (22.0)	39 (19.9)	22 (21.6)	5 (13.2)
Negative	35 (70.0)	150 (76.5)	74 (72.6)	32 (84.2)
Equivocal	3 (6.0)	5 (2.6)	5 (4.9)	1 (2.6)
Missing	1 (2.0)	2 (1.0)	1 (1.0)	0
Median SPT, mm (interquartile range)	3 (3-4)	3 (0-5)	3 (1-5)	4 (2-5)
Reason for Cashew OFC, n (%)				
Not cashew sens (<3mm) or allergic but allergy to other nut/s	4 (8.0)	24 (12.2)	8 (7.9)	2 (5.3)
Not cashew sens (<3mm) or allergic, other non nut allergies	4 (8.0)	12 (6.1)	11 (10.9)	2 (5.3)
Not cashew sens (<3mm) or allergic, no other food allergies	0	16 (8.2)	7 (6.9)	3 (7.9)
Sensitised 3-8mm , never eaten cashew	34 (68.0)	75 (38.3)	36 (35.6)	15 (39.5)
Sensitised >8mm, never eaten cashew	2 (4.0)	11 (5.6)	8 (7.9)	1 (2.6)
Previous cashew reaction	4 (8.0	40 (20.0)	24 (23.8)	13 (34.2)
Reaction to an unknown trigger	1 (2.0)	11 (5.6)	6 (5.9)	1 (2.6)
Unable to determine OFC reason	1 (2.0)	7 (3.6)	1 (1.0)	1 (2.6)
Food Allergy Details, n (%)				
No other food allergies	5 (10.0)	82 (41.8)	37(36.3)	15 (39.5)
Co-existing food allergy				
Peanut allergy	8 (16.0)	37 (18.9)	28 (27.5)	11 (28.9)
Other tree nut [€]	7 (14.0)	21 (10.7)	9 (8.8)	2 (5.6)
Egg allergy	20 (40.0)	27 (13.8)	12 (11.8)	3 (7.9)
Other allergies [ϐ]	10 (25.0)	28 (9.2)	16 (11.7)	7 (17.5)
Allergic disease history, n (%) [¶]				
Current eczema	37 (77.1)	110 (52.3)	47 (47.0)	15 (46.0)
Current asthma	8 (16.6)	76 (39.0)	51 (51.0)	17 (46.0)
Current rhinitis	7.(14.6)	89 (46.3)	72 (72.0)	25 (67.6)

Supplementary Table 2. Cashew OFC outcomes by SPT wheal size, stratified by OFC cohort

SPT wheal size (mm)	Clinical Cohort (RCH/MACCS) n=370		Population Cohort (HealthNuts/SchoolNuts) n=142	
	Cashew Tolerant n (%)	Cashew Allergic n (%)	Cashew Tolerant n (%)	Cashew Allergic n (%)
0	85 (96.6	3 (3.4)	20 (90.9)	2 (9.1)
1	14 (77.8)	4 (22.2)	1(100.0)	0
2	32 (94.1)	2 (5.9)	3 (60.0	2 (40.0)
3	63 (91.3)	6 (8.7)	2 (66.7)	1 (33.3)
4	42 (82.4)	9 (17.6)	9 (100)	0
5	26 (63.4)	15 (36.6)	5 (62.5)	3 (37.5)
6	9 (47.3)	10 (52.6)	1 (28.6)	5 (71.4)
7	3 (42.9)	7 (57.1)	1 (5.9)	13 (94.1)
8	6 (50.0)	7 (50.0)	3 (30.0)	6 (70.0)
9	5 (40.0)	7 (50.0)	1 (16.6)	7 (87.4)
10	1 (25.0)	3 (75.0)	1 (10.0)	9 (90.0)
11	0	1 (100.0)	2 (22.2)	7 (77.8)
12	1 (25.0)	3 (75.0)	0	7 (100.0)
13	0	0	0	6 (100.0)
14	1 (50.0)	1 (50.0)	0	5 (100.0)
15			1 (20.0)	4 (80.0)
16			0	4 (100.0)
17			0	0
18			0	3 (100.0)
19			0	1 (100.0)
20			0	0
21			0	2 (100.0)
22			0	0
23			0	1 (100.0)
24			0	1 (100.0)
Missing	3	0	1	2 (had positive sIgE and negative SPT)

Clinic cohort: n=16 excluded as equivocal OFC outcome
Population cohort: n=3 excluded as equivocal OFC outcome

Supplementary Table 3: Estimated odds ratios of positive OFC for a 1mm increase in SPT wheal size from study-specific logistic regression models.

	Estimate	95% Confidence Interval	P-value for the null hypothesis that the true parameter value is 1.00
RCH	1.50	1.34, 1.69	< 0.001
MACCS	1.74	1.18, 2.56	0.005
HealthNuts	1.44	1.25, 1.66	< 0.001
SchoolNuts	1.67	1.21, 2.32	0.002
Ratio: MACCS to RCH	1.16	0.77, 1.73	0.48
Ratio: SN to HN	1.16	0.81, 1.65	0.41

The coefficients from study-specific logistic regression models of positive OFC on SPT wheal size were estimated. There was no evidence against the null hypothesis that the magnitude of the relationship between positive OFC and SPT wheal size was the same for (1) RCH and MACCS; and (2) HealthNuts and SchoolNuts. In both cases the ratio of estimate log-odds ratios for a 1mm increase in SPT wheal size was 1.16. The p-values for the null hypothesis that the population ratio is 1.00, that is, the relationship is the same for RCH and MACCS, and similarly for HealthNuts and SchoolNuts, are 0.48 and 0.41 respectively.

Supplementary Table 4. Diagnostic capacity of SPTs to challenge confirmed cashew allergy stratified by risk factors for cashew allergy, population cohorts (HealthNuts and SchoolNuts)

Risk Factor	95% PPV mm (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive LR	Negative LR
Sex					
Male (n=84)	13 (7.5-16.0)	22.4 (12.5-35.3)	97.9 (88.9-99.9)	10.8 (1.5-79.3)	0.79 (0.69-0.92)
Female (n=61)	8 (5.35-9.0)	66.7 (51.0-80.0)	96.8 (83.3-99.9)	20.7 (2.9-144)	0.34 (0.23-0.52)
Current food allergy					
Yes (n=75)	9 (7.0-11.0)	58.3 (44.9-70.9)	92 0(78.6-98.3)	7.4 (2.4 (22.4)	0.45 (0.33-0.62)
No (n=65)	13 (7.0-16.0)	20.5 (10.0-36.0)	97.5 (86.8-99.9)	8.2 (1.1-62.6)	0.82 (0.69-0.96)
Type food allergy					
None (n=70)	13 (7.0-16.0)	32.6 (19.1-48.5)	97.3 (85.8-99.9)	8.2 (1.1-62.6)	0.82 (0.69-0.96)
Any nut allergy (n=58)	8 (5.0-10.0)	65.9 (50.0-79.5)	93.1 (77.2-99.2)	9.6 (2.5-37)	0.34)(0.24-0.56)
Any non-nut allergy (n=17)	11 (4.0-16.0)	25 (7.3-52.4)	91.7 (61.5-99.8)	3 (0.4,23.5)	0.82 (0.57,1.14)
Past history of cashew reaction					
Yes (n=23)	Unable to achieve				
No (n=122)	10 (7.5-11.5)	47.7 (36.8-58.7)	95.3 (86.9-99.0)	10.2 (3.3-31.4)	.55 (0.45-0.65)

8. DISCUSSION

Despite the lifelong nature and potential severity of both peanut and tree nut allergy, much of the literature to date has focused on peanut allergy and tree nut allergy remains understudied. This thesis has explored several key areas regarding tree nut allergy in Australia including the prevalence, natural history, frequency of adverse food reactions related to tree nuts and the development of tree nut allergy in the HealthNuts and SchoolNuts population-based cohorts and the Royal Children's Hospital and MACCS allergy clinic populations. A summary of the key findings are outlined below:

8.1 Key Findings

8.1.1 Tree nut allergy prevalence

Worldwide tree nut allergy prevalence

- A systematic review of the literature up until 2014 found tree nut allergy prevalence studies to be limited, with data mainly from Europe, the UK and the USA.
- Prevalence estimates based on challenge-confirmed outcomes for any tree nut were less than 2%.
- Probable tree nut allergy prevalence ranged from 0.05 to 4.9%.
- Prevalence of individual tree nut allergies varied significantly by region with hazelnut the most common tree nut allergy in Europe, walnut and cashew in the USA and Brazil nut, almond and walnut most commonly reported in the UK.
- Australian tree nut allergy prevalence data was limited to one study of self-reported tree nut in children.

Australian tree nut allergy prevalence

- At HealthNuts study recruitment at 12 months of age, (2007-2011) parent-reported tree nut ingestion rates were low (<18%) and parent-reported tree nut allergy rates were less than 1% [0.1%, (95% CI 0.04-0.2)].
- At age 6 years follow up in the HealthNuts study, challenge confirmed tree nut allergy was 3.3% (95% CI 2.8-4.0).
- Cashew was the most common tree nut allergy among those at 6 years of age.

8.1.2 Tree nut allergy adverse events

Among 10 to 14 year olds with food allergy in the SchoolNuts study;

- 44.4% of students with likely IgE-mediated food allergy reported a reaction in the past year (95%CI 40.3, 48.7) and 10% reported reactions consistent with anaphylaxis (95%CI 7.2, 12.2).
- Peanut and tree nut (cashew and walnut) were the most common trigger foods and reactions occurred most frequently at home.
- Those with asthma were two times more likely to report experiencing a food reaction in the past 12 months [aOR 1.9 (95%CI 1.1, 3.2)], however asthma was not associated with experiencing more severe reactions [aOR 1.6 (95% CI 0.7,2.6)].
- Those with multiple food allergies, compared to a single food allergy, were two times more likely to report an adverse food reaction in the past 12 months [aOR 1.9(95% CI 1.1,3.1)] and there was a trend towards reporting more severe reactions [aOR 2.1 (95% CI 0.9,3.8)].
- Those with nut allergies, compared to those without nut allergies, were at decreased risk of reporting a reaction in the past 12 months [aOR 0.7 (95% CI 0.5, 0.1)], however were three times more likely to report a severe reaction [aOR 2.9 (95% CI 1.1,4.4)].

8.1.3 Development of tree nut allergy

In the HealthNuts study;

- Among those with challenge confirmed food allergy at age 1 year, 41% were sensitised to one or more tree nuts.
- Tree nut sensitisation was more common in infants with both peanut and egg allergy (48%) compared to single peanut and (33%) and single egg (24%) allergy.

- Of those children who were sensitized to cashew at age 1 year, 39% had cashew allergy, and 35% were cashew tolerant at age 6years.
- Of children with peanut allergy only at age 1, 27% (95%CI 16.1-39.7) were tree nut allergic at age 6, compared with 14% (95%CI 10.4-17.9) of those with egg allergy only and 37% (95%CI 27.2-47.4) of those with both peanut and egg allergy.

- Of the 147 children with peanut allergy at age 6 years, 45% also had one or more tree nut allergies. The most common tree nut co-allergy for those with peanut allergy at age 6 years was to cashew.

8.1.4 Cashew nut oral food challenge outcomes

Utilising two clinic cohorts and two population-based cohorts of children, the SPT wheal sizes that correlated with 95% predictability of a positive OFC for cashew were determined.

- A cashew SPT wheal size of 10mm or greater (95%CI 7.5-12.0) was found to have 95% PPV to cashew allergy among the population cohort with a positive likelihood ratio of 20.6 (95% CI 2.9 145.2).
- A cashew SPT wheal size of 14mm (95%CI 9.5-unknown) or greater was found to have 95% PPV to cashew allergy among the clinic cohort with a positive likelihood ratio of 10.9 (95% CI 2.2-53.1).

8.2 Strengths and Limitations

The strengths and limitations of the HealthNuts and SchoolNuts studies are discussed in detail in each results chapter. Overall, the strengths of both studies are the large sample sizes, the population sampling frames, high participation fraction and follow-up rates, good internal and external validity, and the utilization of the gold standard test to define food allergy.

Population sampling has ensured that that the full spectrum of food allergy cases and adverse events related to food allergy that would be observed at the community level are captured. Most previous food allergy studies have recruited participants from tertiary allergy centres, which may over represent children with more severe allergic disease resulting in selection bias and generalisability of study findings.

Internal validity of the HealthNuts and SchoolNuts studies was assessed via non-responder questionnaires. For the HealthNuts study, participants were similar to non-participants on most parameters with the exception that participants were more likely to have a family history of food allergy and less likely to have ingested peanut. External validity of the HealthNuts study was assessed by comparing characteristics of the HealthNuts cohort to that of the whole

population using data from the Perinatal Data Collection Unit[4]. For most characteristics, the HealthNuts sample was comparable to the general population. Population sampling, high response rated and good internal and external validity reduce the likelihood of selection bias.

A significant strength of the HealthNuts study is the use of the gold standard OFC to define food allergy outcomes which reduces information bias (misclassification) compared to less robust measures of food allergy, such as self-reporting or tests of IgE sensitisation, which are known to overestimate the prevalence of food allergy.[139] Secondly, to ensure no potential cases of food allergy were missed all participants with a detectable SPT wheal were offered OFC. By challenging all infants with detectable SPT responses, irrespective of the magnitude of SPT response, the classification of food allergy is robust. Participants with negative SPT responses (0mm) were assumed to be food tolerant. To test this assumption, a random sample of 200 participants with negative SPT to all foods underwent OFC; all negative controls tested negative on challenge.

A potential limitation of this research is that both the HealthNuts and SchoolNuts studies utilised open OFCs rather than double-blind, placebo-controlled food challenges. However, only objective symptoms were used to define a positive OFC result, and the validity and sufficiency of using open challenges in infants has been previously confirmed independently.[208]

As with other studies investigating tree nut allergy to date, there were also challenges with determining tree nut allergy outcomes in both the HealthNuts and SchoolNuts studies. At age one recruitment, tree nut SPT were only performed for those participants that attended clinic for an OFC to either milk, egg, sesame or peanut and no OFC were performed for tree nuts. Tree nut SPT was limited to almond, hazelnut and cashew. Therefore, tree nut allergy prevalence estimates at age one are limited to parent-reported reactions. Patterns of sensitisation are limited to the participants that attended clinic and the tree nuts: almond, cashew and hazelnut.

At age 6 years tree nut allergy prevalence estimates were possible as tree nut SPT were performed on the entire cohort and as per age one protocol, all sensitised participants offered an OFC. However, not all study participants had SPT or nut consumption data available at age 6 years. Almond, cashew and hazelnut had more complete screening and there is limited SPT and OFC for the additional tree nuts. Most of those deemed allergic to Brazil nut, macadamia,

pecan, pistachio and walnut are based on high-level sensitisation (SPT>8mm) not OFC and many of those with mid-range sensitisation (3-7mm) did not have OFC performed. This was largely due to the high number of participants with sensitisation to multiple tree nuts and the burden to families and study resources to perform multiple OFC for the one participant. This limits the quality of the data for exploring the co-allergy patterns reported and is an important factor to consider for tree nut allergy studies in the future. In addition, there is also likely to be an allergic bias in participation and follow up at age 6 as we observed participants with a family history of allergy and children with eczema or food allergy at age 1 year were more likely to participate in the follow-up at age 6 years. Therefore we have reported a range of more and less conservative observed prevalence estimates and a population prevalence estimate for tree nut allergy re-weighted for factors that were associated with participation at age 6 years.

The SchoolNuts study was limited by the use of self-reported data regarding adverse food reactions however the use of both student and parent questionnaires allowed validation of self-reported responses for food allergy, asthma and allergic reaction details. Kappa analysis showed strong correlation between student and parent dyad responses for self-reported food allergy. Additionally, whilst clinical samples have the strength of more robust diagnosis and definition of adverse reactions and anaphylaxis compared to self-reported reactions they risk missing those experiencing milder reactions and over representing those with more severe food allergy phenotypes, higher socio-economic status and those with greater access to specialised medical management and education which are captured with a population-based study.

Finally, PPVs are dependent on the underlying prevalence of disease, therefore thresholds developed in one setting may not be generalisable to another setting if the prevalence of food allergy is substantially different. This can be overcome by the use of likelihood ratios that are presented alongside all PPVs reported in this thesis.

8.3 Implications of this work

Until recently tree nuts have been an under considered and understudied food allergen. This body of work has reported the first challenge confirmed tree nut allergy prevalence rates in Australia, determining one of the highest reported tree nut allergy rates in the world to date, with cashew the most common individual tree nut allergy. This work has highlighted the regional variability of overall and individual tree nut allergy prevalence rates and the need for region specific studies that include all the common culinary nuts.

Confirming other reports of adverse food reactions,[147,151-153] the work conducted as part of this PhD has found peanut and tree nut the most common triggers of adverse food reactions for children aged 10 to 14 years, with cashew the most common tree nut trigger reported. Certainly based on this work cashew appears to be equal to peanut in prevalence and severity of reactions in Melbourne, Australia.

This work has also highlighted some important considerations around tree nut allergy prevention. Recent systematic review and meta-analysis evidence has shown that early peanut or egg introduction reduces the risk of a child developing peanut or egg allergy[9]. This has resulted in a paradigm shift in clinical practice, from advice to delay the introduction of peanut and egg to actively encouraging introduction before 12 months of age. Accordingly, infant feeding guidelines (in Australia and internationally)[30,209-211] have been revised to recommend the introduction of peanut and other allergenic solids in the first year of life. Whether timely introduction of tree nuts in infancy promotes immune tolerance and provides protection from developing tree nut allergy is not known. Work as part of this thesis has shown that among those with co-existing food allergy at 12 months of age, sensitisation rates to tree nut were as high as 48% and in the context of allergen avoidance for the sensitised tree nuts, 30% of those tree nut sensitised at 12 months were tree nut allergic at 6 years of age. With such high levels of tree nut sensitisation among infants with food allergy, tree nut SPT screening has the potential to impose a significant burden on allergy clinics to confirm allergy status for each individual tree nut. Evidence that tree nut allergy can be prevented is needed before making recommendations to identify and target children at high risk of tree nut allergy early in life. Further research is required to guide tree nut introduction advice for those at high risk of tree nut allergy.

There remains significant limitations to food allergy diagnosis with a high dependency on time consuming, costly and potentially risky OFCs to achieve a definitive diagnosis. Current waiting lists at the Royal Children's' Hospital allergy clinic for food challenges are lengthy and OFC are prioritised for core foods such as milk, egg and wheat. Many children sensitised to tree nuts are not able to have an OFC performed and are often avoiding multiple tree nuts based on IgE sensitisation status alone. The future of food allergy diagnosis lies in the use of multiple diagnostic parameters offering greater precision than IgE testing alone which may eradicate the need for OFC. There are very promising technical advances on the horizon such as basophil activation testing and improvement in cellular assays and component-resolved diagnostics but until they are ready for clinical application the use of 95% PPVs for SPT and sIgE remain a useful adjunct to clinical decision making regarding a patient's need for an OFC. To date, SPT thresholds for tree nut have not been reported and clinicians often apply a generic 8mm threshold which is not specific to any of the individual tree nuts nor the Australian population. The cashew SPT threshold generated from this PhD will improve the management of cashew allergy (the most common tree nut allergy in Australia) by providing a reliable SPT threshold based on an Australian population-based cohort. It is hoped this can assist with identifying those children needing an OFC and reduce food challenge waiting lists and consequently improve access to tertiary care for children with complex allergies.

8.4 Future directions

The work as part of this PhD has outlined the burden of tree nut allergy in Australian children and provides an important foundation for further work regarding tree nut allergy prevention, diagnosis and management.

It is postulated that food allergy, including tree nut allergies are increasing but there are limited data on food allergy time trends, particularly for tree nut allergy. The EarlyNuts study aims to recruit 2,000 infants aged 12 months from immunisation sessions across Melbourne. With the same study methods as the HealthNuts study, Wave 1 which was completed 10 years ago, the EarlyNuts study aims to establish if there has been a change in food allergy prevalence among Australian infants. It will also provide information regarding the timing of introduction of allergenic foods, including tree nuts. Preliminary results suggest common allergens such as peanut, egg and cashew are being introduced earlier for a larger proportion of infants in the first 12 months of life than in the HealthNuts study 10 years ago. Unlike HealthNuts, EarlyNuts

There is currently no data on the primary or secondary prevention of tree nut allergy, therefore further research is planned to explore the impact of early introduction of tree nuts in high risk children through a randomised controlled trial in the RCH Allergy clinic population of which I am an associate investigator, called the TreEAT study. We hypothesize that optimal introduction of tree nuts in the first year of life will reduce childhood tree nut allergy. We also hypothesize that a supervised multi-nut OFC in high risk infants will improve the rates of tree nut introduction and ongoing ingestion at home, ultimately lowering the rate of tree nut co-sensitisation and challenge-proven allergy. We have designed a randomised, open-label controlled trial for infants (aged 6 to 12 months) at high risk of tree nut allergy. We specifically aim to determine the efficacy of a multi-tree nut OFC compared to recommending home introduction (without prior supervised OFC) to (1) reduce challenge-proven tree nut co-allergy at 18 months (primary objective), (2) reduce tree nut sensitisation at 18 months of age, and (3) improve rates of home tree nut ingestion at 18 months. This study will be the first in the world to provide evidence for a management guideline for the prevention of tree nut allergy in high-risk infants (peanut and/or egg allergic). Within the next 4 years, clinical outcomes from this randomized controlled trial will provide evidence to inform clinical practice in primary and secondary prevention of tree nut allergy from infancy.

As part of this thesis a 95% PPV threshold for cashew SPT was determined and work is underway to establish 95% PPV thresholds for SPT for additional tree nuts. Other work that is planned for improving the diagnosis of tree nut allergy is a trial of basophil activation testing in the TreEat RCT which has shown promise for improved diagnosis for other allergens but studies for tree nut allergens and clinical application has to date been limited.

Our understanding of tree nut allergy time trends and natural history is limited due to the paucity of longitudinal tree nut allergy studies and current data is based on tertiary level allergy clinic populations. Ongoing research within the HealthNuts study includes all 5276 participants being contacted at 10 years of age and invited to undergo a detailed health assessment including SPT to food and inhalant allergen, OFCs if food sensitised, lung function testing, retinol photography, physical assessments and collection of biological specimens (blood and cheek swabs) for immunological, genetic and epigenetic studies. This will allow further investigation of the natural history of tree nut allergy and identification of modifiable risk factors which may influence the development of tree nut allergy and prevent progression

to multiple food allergies, within a population-based cohort with challenge confirmed allergy outcomes.

The pinnacle of food allergy research is a treatment with most of the work focusing on systemic epicutaneous, sublingual and oral immunotherapy.[64] To date this research has been limited for tree nut allergy but work as part of this thesis has highlighted cashew as a priority tree nut for expansion of the work already underway for peanut allergy oral immunotherapy.[75,76,212-219]

8.5 Conclusion

Within this thesis, I have examined several key areas regarding tree nut allergy in Australia including the prevalence, frequency of adverse food reactions related to tree nuts and elements of the natural history and development of tree nut allergy in population-based HealthNuts and SchoolNuts cohorts and the Royal Children's Hospital and MACCS allergy clinic populations.

These findings have made a significant contribution to the literature and shaped the future direction of many of the epidemiological, clinical and laboratory based research projects within our own research group and those of our collaborators and has immediate implications for the clinical management of tree nut allergy.

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Appendix 1. HealthNuts Wave 1 questionnaire

BLACK Raspberry

Murdoch Childrens Research Institute

ID ☐☐☐☐ DOQ D D / M M / Y Y Y Y

HealthNuts : How does food allergy affect our community?

It is important that you have read the information sheet and have your questions answered before signing your consent form. Have you signed your consent form?
If not, please do this now before completing this questionnaire.
There are no right or wrong answers. For most questions, there is a choice of answers.
Pick the one that's true for you and cross the box next to it like this: Yes ☒
Please cross ONE box only unless otherwise requested. If you make a mistake, simply scribble it out and mark the correct answer with a cross: like this: No ☒ Yes ☒
Some questions ask you to write a short answer in the space provided.
Use a ballpoint blue or black pen (do NOT use a felt tipped pen).

– Let's start!

Child's Name: ☐ ☐ ☐

Child's Date of birth: D D / M M / Y Y Y Y Sex: Male ☐ Female ☐

Your Name: ☐ ☐ ☐

Your relationship to the child: ☐

Address: ☐ Postcode: 3 ☐☐☐

Phone: Mobile ☐☐☐☐☐☐☐☐☐☐ Home (☐☐) ☐☐☐☐☐☐☐☐

QUESTIONS ABOUT YOUR CHILD

1. How was your child born?
   Vaginal ☐ Caesarean section ☐
2. At how many weeks gestation did you deliver this baby?
   other | 36 | 37 | 38 | 39 | 40 | 41↑ | 42 | other
3. Birth weight: ☐ g
4. Birth Length: ☐ cm
5. Last measured weight: ☐ g
   Date D D / M M / Y Y Y Y
6. Last measured length: ☐ cm
   Date D D / M M / Y Y Y Y

FEEDING YOUR CHILD

7. Has your child ever had a reaction (e.g. redness or itching) which you thought was due to some food that they had eaten?
   No ☐ Yes ☐
   → If no go to Q.12
8. Was there more than one type of food?
   No ☐ Yes ☐
9. What was the food(s) (e.g. peanut) ☐
10. How long after the food was eaten did the reaction appear?
    < ½ hr ☐ 1-4 hrs ☐ > 4 hrs ☐
11. Describe the reaction(s)
    skin rash ☐ difficulty breathing ☐
    vomiting ☐ other *(describe)* ☐
    diarrhoea ☐ ☐

BLACK Raspberry

We are now going to ask you questions on the introduction of food to your child's diet.
(please circle* weeks *or* months *)

12. Age started breastfeeding (include colostrum in the first few days after birth)

Age ☐ in days

Not sure ☐
Not started ☐

13. Still breastfed

No ☐ Yes ☐

14. Age breastfeeding stopped

Age ☐ weeks / months

Not sure ☐
Not started ☐

15. Age infant formula bottle feeding started

Age ☐
weeks / months

Not sure ☐
Not started ☐

16. Age infant formula bottle feeding stopped

Age ☐ weeks / months

Not sure ☐
Not started ☐

17. When was solid food first introduced

Age ☐ weeks / months

Not sure ☐
Not started ☐

18a. Age of change from formula to cow's milk

Age ☐ weeks / months

Not sure ☐
Not started ☐

19. Brand(s) of formula used and age introduced (See flash card of labels in folder)

Brand ☐
Age ☐ months

Brand ☐
Age ☐ months

20. Has your child eaten ..... Nuts?

	No	Don't know	Yes
Peanut butter	☐	☐	☐
Peanut oil	☐	☐	☐
Other nuts (e.g. cashews, mixed nuts) *(please specify)*	☐	☐	☐

21. Has your child consumed ..... Eggs?

	No	Don't know	Yes
Soft boiled / scrambled egg	☐	☐	☐
Hard boiled egg	☐	☐	☐
Meringue	☐	☐	☐
Cakes	☐	☐	☐
Biscuits	☐	☐	☐
Other form *(Please Specify)*	☐	☐	☐

22. Has your child consumed ..... Other foods?

	No	Don't know	Yes
Sesame product	☐	☐	☐
Tahini	☐	☐	☐
Sesame seeds on bread	☐	☐	☐
Fish	☐	☐	☐
Shellfish *(Please Specify)*	☐	☐	☐

23. Has your child consumed ..... Soy (not including formula)?

	No	Don't know	Yes
Soy milk or soy products *(Please Specify)*	☐	☐	☐

YOUR CHILD'S RASHES

24. Has you child at any time had an itchy rash other than nappy rash?

No ☐ Yes ☐

→ If no go to 0.29

25. Did you use medication to treat it?

Never D
Used in the past D
Used now D
Not sure D

26. Creams or moisturizers?

No D Yes D Notsure O

Name

27. Topical Steroids (e.g. cortisone)

No D Yes D Not sure O

Name

28. Did you use steroids for more than 10 days in a row?

No D Yes D Not sure O

29. Has your child ever been diagnosed with eczema?

No ☐ Yes ☐ Not sure ☐

30. Age when eczema was first diagnosed?

months old

31. Has your child ever wheezed?

No ☐ Yes ☐ Not sure ☐

32. Number of episodes of wheeze?

33. Has your child ever had bronchiolitis (bron-key-o-litus)

No ☐ Yes ☐ Not sure ☐

34. Was your child hospitalised with bronchiolitis?

No ☐ Yes ☐ Not sure ☐

If yes, age ☐ in months

35. Has your child ever had antibiotics?

No ☐ Yes ☐ Not sure ☐

If yes, age ☐ in months

Reason (e.g. ear infection)

36. Type of antibiotic (if known)

37. If more than one course of antibiotic, how many?

number
number
number

38. Does your child attend childcare / daycare?

No ☐ Yes ☐

Does your child attend family care?

No ☐ Yes ☐

How many days per week?

1	2	3	4	5	6	7

How many hours per session?

1	2	3	4	5	6	7	8	9	10	11	12

At what age did they begin (months old)?

1	2	3	4	5	6	7	8	9	10	11	12

39. In general, would you say your child's health is *(please cross one box)*...

Excellent D
Very good D
Good D
Fair D
Poor D

BLACK Raspberry

40. Compared to other babies, I think my baby is *(please cross one box):*

- Much easier than average ☐
- Easier than average ☐
- Average ☐
- More difficult than average ☐
- Cannot say ☐

41. Do you believe your child has a food allergy?

No ☐ Yes ☐ Not sure ☐

42. Do you believe your child is at risk of food allergy?

No ☐ Yes ☐ Not sure ☐

43. Has your child ever had colic?

No ☐ Yes ☐ Not sure ☐

•11 no go to Q44

If yes. how old was the child when it started?

☐ weeks / months

How many hours per day on average did they have colic?

☐

How long did it last?

☐ days / weeks / months

Did you consult a doctor?

No ☐ Yes ☐ Not sure ☐

Did you change your child's formula?

No ☐ Yes ☐ Not sure ☐

Was your child hospitalised?

No ☐ Yes ☐ Not sure ☐

44. Did your child ever have reflux?

No ☐ Yes ☐ Not sure ☐

•11 no go to Q45

If yes. how old was the child when it started?

☐ weeks / months

How long did it last?

☐ days / weeks / months

Did you consult a doctor?

No ☐ Yes ☐ Not sure ☐

Did you change your child's formula?

No ☐ Yes ☐ Not sure ☐

Was your child hospitalised?

No ☐ Yes ☐ Not sure ☐

Was your child prescribed medication?

No ☐ Yes ☐ Not sure ☐

Which medication? ☐

45. Did your child ever suffer from bouts of vomiting?

No ☐ Yes ☐ Not sure ☐

•11 no go to Q46

If yes, how old was the child when it started?

☐ weeks / months

How long did it last?

☐ days / weeks / months

Did you consult a doctor?

No ☐ Yes ☐ Not sure ☐

Did you change your child's formula?

No ☐ Yes ☐ Not sure ☐

Was your child hospitalised?

No ☐ Yes ☐ Not sure ☐

46. Did your child ever suffer from diarrhoea?

No ☐ Yes ☐ Not sure ☐

•11 no go to Q47

If yes, how old was the child when it started?

☐ weeks / months

How long did it last?

☐ days / weeks / months

Did you consult a doctor?

No ☐ Yes ☐ Not sure ☐

Did you change your child's formula?

No ☐ Yes ☐ Not sure ☐

Was your child hospitalised?

No ☐ Yes ☐ Not sure ☐

47. Cross the foods that the child's [illegible] has eaten.....

Which food?	When? (you can cross more than one)				How often?		
	Never eat food	Ate food during pregnancy	Ate food while breastfeeding	Not sure	Less than once a week	At least once a week	Daily
Nuts							
Peanut (e.g roasted, peanut butter, cakes)	☐	☐	☐	☐	☐	☐	☐
Any other nuts e.g. cashews, walnuts *(please specify)*	☐	☐	☐	☐	☐	☐	☐
Other foods							
Sesame products	☐	☐	☐	☐	☐	☐	☐
Shellfish	☐	☐	☐	☐	☐	☐	☐
Eggs	☐	☐	☐	☐	☐	☐	☐
Soy or soy products	☐	☐	☐	☐	☐	☐	☐

48. Have you had any supplements or drugs during your pregnancy?

	No	Yes	Not sure
Iron	☐	☐	☐
Folate	☐	☐	☐
Multivitamin	☐	☐	☐
Fish Oil	☐	☐	☐
Calcium	☐	☐	☐
Probiotics	☐	☐	☐
Alternative medicine supplement *(please describe)*	☐	☐	☐
Other *(please describe)*	☐	☐	☐
Drug Treatments (please describe)	☐	☐	☐

BLACK Raspberry

QUESTIONS ABOUT YOUR FAMILY

49. Does your 12 month old have other brothers and sisters?

No ☐ Yes ☐

Date of birth

Brother 1 ☐☐/☐☐/☐☐☐☐

Brother 2 DD/DD/DODD

Sister 1 DD/DD/DODD

Sister2 DD/DD/DODD

other siblings DDID DIDO DD

50. Mother's date of birth

DD/DD/DODD

5'. Father's date of birth

DD/DD/DODD

52. The following people live with my child in our house (for at least half the week) the child's.....?

Father ☐

Mother D

Siblings (as described above) ☐

Other relatives(describe e.g. uncles, grandmother) ☐

Other people (describe eg, friend of family, lodger) ☐

53. In what country was the child's mother born?

Australia ☐

Other ☐
(please list)

54. In what country was the child's father born?

Australia ☐

Other
(please list)

55. What is the main language spoken at home?

English ☐
Italian ☐
Greek ☐
Vietnamese ☐
Arabic ☐
Turkish ☐
Chinese ☐
Other *(please list)* ☐

56. Have you moved to Australia from another country in the last 5 years.

No ☐ --+ If no go to Q59
Yes ☐ --+ Which country?

57. Has your diet changed significantly since moving to Australia?

Strongly agree ☐
Agree ☐
Not sure ☐
Disagree ☐
Strongly Disagree ☐

58. Which ONE or MORE of the following statement best applies to your diet since moving to Australia?

- My diet is much the same now as before the move ☐
- I eat MORE processed food now than before (eg foods that are bought in a packet) ☐
- I eat LESS processed food now than before (eg foods that are bought in a packet) ☐
- I eat MORE take-away food and restaurant food now than before (eg hamburgers, fish and chips) ☐
- I eat LESS take-away food and restaurant food now than before (eg hamburgers, fish and chips) ☐

59. Does anyone smoke inside the home?

No ☐ Yes ☐

Who? _____ Number _____ cigs/day

60. Does anyone smoke outside the home (e.g. in the garden)?

No ☐ Yes ☐

Who? _____ Number _____ cigs/day

61. Did the mother smoke in pregnancy?

No ☐ Yes ☐

Number _____ cigs/day

62. Did the mother smoke in the past?

No ☐ Yes ☐

How long _____ yrs Number _____ cigs/day

63. Did the father smoke in the past?

No ☐ Yes ☐

How long _____ yrs Number _____ cigs/day

BLACK Raspberry

64. Does anyone in your family suffer.....*(please cross)*?

	1 year old	Mother	Father	Brother 1	Brother 2	Sister 1	Sister 2	Other bro	Other sis
Asthma	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dermatitis	☐	☐	☐	☐	☐	☐	☐	☐	☐
Eczema	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hay fever	☐	☐	☐	☐	☐	☐	☐	☐	☐
Seasonal allergies	☐	☐	☐	☐	☐	☐	☐	☐	☐
Year round allergies	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sinus problems	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lupus	☐	☐	☐	☐	☐	☐	☐	☐	☐
Arthritis	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other allergies :									
Bee sting / ants	☐	☐	☐	☐	☐	☐	☐	☐	☐
Drug	☐	☐	☐	☐	☐	☐	☐	☐	☐
Food *(please specify)*	☐	☐	☐	☐	☐	☐	☐	☐	☐
Latex	☐	☐	☐	☐	☐	☐	☐	☐	☐
Nickel jewellery	☐	☐	☐	☐	☐	☐	☐	☐	☐

65. Could you please give an indication of your combined household income (yearly)?

Our household income is

$

or

- 0-25,000 ☐
- 25,000-50,000 ☐
- 50,000-75,000 ☐
- 75,000-100,000 ☐
- More than 100,000 ☐
- Don't know ☐
- Don't wish to answer question ☐

66. Do you have pets at home?

	Inside	Outside	Both
Cat	☐	☐	☐
Dog	☐	☐	☐
Bird	☐	☐	☐
Other *(please describe)*	☐	☐	☐

67. Do you live on a farm with animals?

No ☐ Yes ☐

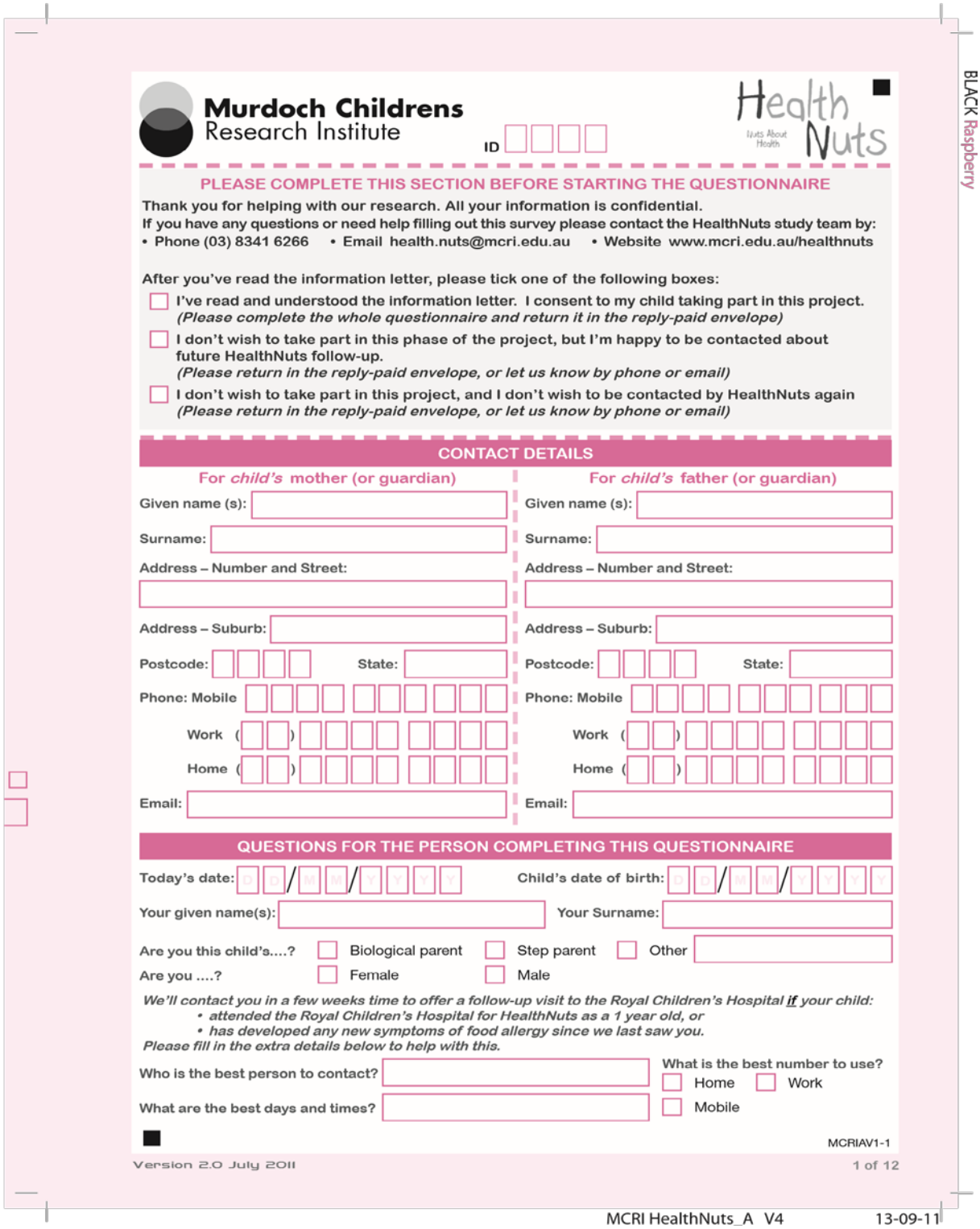
Thank you for participating in our study.
Please let the researcher know you have finished filling out the questionnaire and they will help you with the rest of the allergy test.
HealthNuts – Nuts about health

Murdoch Childrens Research Institute

Version 5.0 January 2008

MCRI HealthNuts V3-Final 27-06-08

Appendix 2. HealthNuts Age 4 Questionnaire



Murdoch Childrens Research Institute

ID

HealthNuts — Nuts About Health

PLEASE COMPLETE THIS SECTION BEFORE STARTING THE QUESTIONNAIRE

Thank you for helping with our research. All your information is confidential.
If you have any questions or need help filling out this survey please contact the HealthNuts study team by:
• Phone (03) 8341 6266 • Email health.nuts@mcri.edu.au • Website www.mcri.edu.au/healthnuts

After you've read the information letter, please tick one of the following boxes:

- ☐ I've read and understood the information letter. I consent to my child taking part in this project.
  (Please complete the whole questionnaire and return it in the reply-paid envelope)
- ☐ I don't wish to take part in this phase of the project, but I'm happy to be contacted about future HealthNuts follow-up.
  (Please return in the reply-paid envelope, or let us know by phone or email)
- ☐ I don't wish to take part in this project, and I don't wish to be contacted by HealthNuts again
  (Please return in the reply-paid envelope, or let us know by phone or email)

CONTACT DETAILS

For *child's* mother (or guardian)	For *child's* father (or guardian)
Given name (s):	Given name (s):
Surname:	Surname:
Address – Number and Street:	Address – Number and Street:
Address – Suburb:	Address – Suburb:
Postcode: State:	Postcode: State:
Phone: Mobile	Phone: Mobile
Work ( )	Work ( )
Home ( )	Home ( )
Email:	Email:

QUESTIONS FOR THE PERSON COMPLETING THIS QUESTIONNAIRE

Today's date: D D / M M / Y Y Y Y Child's date of birth: D D / M M / Y Y Y Y

Your given name(s): Your Surname:

Are you this child's....? ☐ Biological parent ☐ Step parent ☐ Other

Are you ....? ☐ Female ☐ Male

*We'll contact you in a few weeks time to offer a follow-up visit to the Royal Children's Hospital **if** your child:*
- *attended the Royal Children's Hospital for HealthNuts as a 1 year old, or*
- *has developed any new symptoms of food allergy since we last saw you.*

Please fill in the extra details below to help with this.

Who is the best person to contact?

What are the best days and times?

What is the best number to use?
☐ Home ☐ Work
☐ Mobile

MCRIAV1-1

Version 2.0 July 2011 1 of 12

BLACK Raspberry

Raspberry

1 FEEDING YOUR CHILD

We last saw you when your child was 12 months old. We want to know what new foods you've introduced into your child's diet since that time. Since age 12 months, has your child eaten the following foods? (tick one box on each line)

		No	Eaten 3 times or less	Eaten more than 3 times
1.1	Peanut butter	D	D	D
1.2	Peanuts	D	D	D
1.3	Pistachios	D	D	D
1.4	Cashews	D	D	D
1.5	Almonds	D	D	D
1.6	Hazelnuts (Including Nutella)	D	D	D
1.7	Pine nuts	D	D	D
1.8	Other nuts *(please specify)*	D	D	D
1.9	Tahini (or hummus)	D	D	D
1.10	Sesame seeds on foods (eg. bread, sesame snaps)	D	D	D
1.11	Semi-cooked (runny) egg (eg. scrambled, soft boiled, fried, poached)	D	D	D
1.12	Completely cooked (hard) egg (eg. hard boiled, fried, poached)	D	D	D
1.13	Meringue, pavlova or macaroons	D	D	D
1.14	Cakes containing egg	D	D	D
1.15	Biscuits containing egg (eg teddy bear biscuits)	D	D	D
1.16	Other foods containing egg (please *specify)*	D	D	D
1.17	Fish	D	D	D
1.18	Shellfish *(please specify)*	D	D	D
1.19	Soy milk or other soy products *(please specify)*	D	D	D
1.20	Cow's milk (including on cereal)	D	D	D
1.21	Cow's milk in baked products (eg. cakes, muffins)	D	D	D
1.22	Other dairy products (e.g. cheese, yoghurt, cream, ice cream) *(please specify)*	D	D	D
1.23	Other types of milk e.g. goat's milk *(please specify)*	D	D	D
1.24	Wheat (e.g. bread, cakes, biscuits)	D	D	D

1.25 Do you restrict any particular foods In your child's diet? 0 No Oves

If yes, (a) which foods does your child avoid?

(b) why are these foods avoided?

1.26 At what age did this child stop breastfeeding? [_____] months (write NIA if never breastfed)

Murdoch Childrens Research Institute

Version 2.0 July 2011

Raspberry

2.2 Have you ever consulted any health professionals about your child's food reactions?

No ☐ ---→ If no, go to Question 3.1

a.	Doctor - general practitioner (GP)	☐ How many times did you see a GP?	
b.	Paediatrician	☐ How many times did you see a paediatrician?	
c.	Allergist	☐ How many times did you see an allergist?	
d.	Other health professional *(please specify)*	☐ How many times did you see this health professional?	
e.	Complementary medicine practitioner *(please specify)*	☐ How many times did you see a complementary medicine practitioner?	

2.3 Does your child currently have an EpiPen/Anapen for **food allergy**? ☐ No ☐ Yes

3. RASHES, CHEST SYMPTOMS, RUNNY NOSE

3.1 In general, would you say your child's health is:

☐ Excellent ☐ Very good ☐ Good ☐ Fair ☐ Poor

Rashes

3.2 Has your child ever had an itchy rash which was coming and going for at least six months?

☐ No ---→ go to Question 3.3 ☐ Yes ☐ Don't know

If yes...

a. Has your child had this itchy rash at any time in the last 12 months?
☐ No ☐ Yes ☐ Don't know

b. Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?
☐ No ☐ Yes ☐ Don't know

c. At what age did this itchy rash first occur?
☐ Under 2 years ☐ Age 2-4 years

d. Has this rash cleared completely at any time in the last 12 months?
☐ No ☐ Yes ☐ Don't know

e. In the last 12 months, how often, on average, has your child been kept awake at night by this itchy rash?
☐ Never in the last 12 months
☐ Less than one night per week
☐ One or more nights per week

Raspberry

3.3 Has your child been diagnosed with eczema?

☐ No ☐ Yes ---→ a. Age when symptoms started ☐ months old

3.4 In the last 12 months, has your child suffered from dry skin in general?

☐ No ☐ Yes

3.5 Did you use medication to treat your child's eczema, itchy rash or dry skin?
(You can tick more than one box if necessary)

☐ Never ---→ go to Question 3.6 ☐ Used in the past ☐ Use now

If you have ever used medication for your child's eczema or itchy rash

a. Have you used moisturisers?
☐ No ☐ Yes Name ☐ Don't know

b. Have you used topical steroid creams or ointments (e.g. sigmacort, celestone, elocon, cortic, hydrocortisone, advantan fatty ointment)?
☐ No ☐ Yes Name ☐ Don't know

c. Did you use steroid creams for more than 10 days in a row?
☐ No ☐ Yes ☐ Don't know

3.6 Have you ever consulted any health professionals about your child's eczema or dry skin?

No ☐ ---→ If no, go to Question 3.7

a.	Doctor - general practitioner (GP)	☐ How many times did you see a GP?	☐
b.	Paediatrician	☐ How many times did you see a paediatrician?	☐
c.	Allergist	☐ How many times did you see an allergist?	☐
d.	Other health professional *(please specify)* ☐	☐ How many times did you see this health professional?	☐
e.	Complementary medicine practitioner *(please specify)* ☐	☐ How many times did you see a complementary medicine practitioner?	☐

Wheezing and coughing

3.7 Has your child ever had wheezing or whistling in the chest at any time in the past?

☐ No ---→ go to Question 3.8 ☐ Yes ☐ Don't know

If yes...

a. At what age did the symptoms first start? ☐ year(s) old

b. Has your child had wheezing or whistling in the chest in the last 12 months?
☐ No ---→ go to Question 3.8 ☐ Yes

c. How many attacks of wheezing has your child had in the last 12 months?
☐ None ☐ 1 to 3 ☐ 4 to 12 ☐ More than 12

Raspberry

d. In the last 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?

- ☐ Never woken with wheezing
- ☐ Less than one night per week
- ☐ One or more nights per week

e. In the last 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths?

☐ No ☐ Yes

3.8 Has your child ever had asthma?

☐ No

☐ Yes ---→ a) Were you told by a doctor that your child had asthma? ☐ No ☐ Yes

b) Age when symptoms started ☐ years old

3.9 In the last 12 months has your child used any medicines, pills, puffers or other medications for wheezing or asthma?

☐ No ---→ **go to Question 3.10** ☐ Yes ☐ Not sure

If yes, please list the medications and when they were used.

a. Name of 'Western' medicine	When wheezy	Regularly (every day for at least 2 months)
☐	☐	☐
☐	☐	☐
☐	☐	☐
b. Name of 'Alternative' medicine		
☐	☐	☐
☐	☐	☐

3.10 Have you ever consulted any health professionals about your child's wheezing or asthma?

No ☐ ---→ **If no, go to Question 3.11**

a.	Doctor - general practitioner (GP)	☐ How many times did you see a GP?	☐
b.	Paediatrician	☐ How many times did you see a paediatrician?	☐
c.	Allergist	☐ How many times did you see an allergist?	☐
d.	Other health professional *(please specify)* ☐	☐ How many times did you see this health professional?	☐
e.	Complementary medicine practitioner *(please specify)* ☐	☐ How many times did you see a complementary medicine practitioner?	☐

Raspberry

Hay fever

*All questions are about problems which occur when your child **DOES NOT** have a cold or the flu.*

3.11 **Has your child ever had sneezing or a runny or blocked nose, when he/she did not have a cold or the flu?**

☐ No ----→ go to Question 3.12 ☐ Yes ☐ Don't know

If yes...

a. At what age did the symptoms first start? ☐☐ year(s) old

b. In the past 12 months, has your child had a problem with sneezing or a runny or blocked nose when he/she DID NOT have a cold or the flu?

☐ No ----→ go to Question 3.12 ☐ Yes ☐ Don't know

c. In the past 12 months, has this problem been accompanied by itchy-watery eyes?

☐ No ☐ Yes ☐ Don't know

d. In the past 12 months, how much did this nose problem interfere with your child's activities?

☐ Not at all ☐ A little ☐ A moderate amount ☐ A lot

e. In which of the past 12 months did nose problems occur?
(tick all that apply)

☐ January	☐ April	☐ July	☐ October
☐ February	☐ May	☐ August	☐ November
☐ March	☐ June	☐ September	☐ December

3.12 **Has your child ever had hay fever?**

☐ No ----→ go to Question 4.1

☐ Yes ----→ a. Were you told by a doctor that your child had hay fever? ☐ No ☐ Yes

b. Age when symptoms started ☐☐ years old

3.13 **Have you ever consulted any health professionals about your child's hay fever?**

No ☐ ----→ If no, go to Question 4.1

a.	Doctor - general practitioner (GP)	☐ How many times did you see a GP?	☐☐
b.	Paediatrician	☐ How many times did you see a paediatrician?	☐☐
c.	Allergist	☐ How many times did you see an allergist?	☐☐
d.	Other health professional *(please specify)* ☐☐☐☐☐☐☐☐☐☐	☐ How many times did you see this health professional?	☐☐
e.	Complementary medicine practitioner *(please specify)* ☐☐☐☐☐☐☐☐☐☐	☐ How many times did you see a complementary medicine practitioner?	☐☐

Raspberry

4. OTHER QUESTIONS ABOUT YOUR CHILD

4.1 Does your child regularly receive childcare or go to kindergarten or preschool?

☐ No ---→ go to Question 4.2

☐ Yes ---→ a. On average, how many hours does your child spend there every week?

☐☐ hrs /week

b. At what age did your child start childcare? ☐☐ months

c. At what age did your child start kindergarten/preschool? ☐☐ months

d. How many children does your child have contact with at childcare, kindergarten or preschool?

☐ 1-5 ☐ 5-9 ☐ 10-15 ☐ more than 15

4.2 Has your child ever had a gastrointestinal worm infection (e.g. Threadworms or Pinworms)

☐ No ---→ go to Question 4.3

☐ Yes ---→ a. How old was your child when they first had worms?

☐ Less than 1 year ☐ 1 to 3 years ☐ At least 3 years

b. How many times have they had worms? ☐☐

4.3 During the last year, how much time did your child spend in the sun?

	Summer	<1 hr a day	1 to 2 hrs per day	2 to 3 hrs per day	3 to 4 hrs per day	≥ 4 hrs a day
a.	on weekdays	☐	☐	☐	☐	☐
b.	on weekends	☐	☐	☐	☐	☐
	Winter					
c.	on weekdays	☐	☐	☐	☐	☐
d.	on weekends	☐	☐	☐	☐	☐

4.4 a. What was your child's last measured **weight**? ☐☐.☐ kg

b. Date recorded D D / M M / Y Y

4.5 a. What was your child's last measured **height**? ☐☐☐ cm

b. Date recorded D D / M M / Y Y

4.6 Does your child have **younger** brothers and sisters? (we already know about older siblings from our previous questionnaire)

☐ No younger siblings ☐ Yes *(please give details below)*

Younger sisters	Date of birth	Younger brothers	Date of birth
a. Sister 1	D D / M M / Y Y	b. Brother 1	D D / M M / Y Y
c. Sister 2	D D / M M / Y Y	d. Brother 2	D D / M M / Y Y
e. Sister 3	D D / M M / Y Y	f. Brother 3	D D / M M / Y Y

4.7 Below is a list of things that might be a problem for your child. Please tell us how much of a problem each one has been for your child in the past ONE month.

		Never	Almost Never	Some-times	Often	Almost Always
	Physical functioning (problems with…)					
a.	Walking	☐	☐	☐	☐	☐
b.	Running	☐	☐	☐	☐	☐
c.	Participating in active play or exercise	☐	☐	☐	☐	☐
d.	Lifting something heavy	☐	☐	☐	☐	☐
e.	Bathing	☐	☐	☐	☐	☐
f.	Helping to pick up his or her toys	☐	☐	☐	☐	☐
g.	Having hurts or aches	☐	☐	☐	☐	☐
h.	Low energy levels	☐	☐	☐	☐	☐
	Emotional functioning (problems with...)					
i.	Feeling afraid or scared	☐	☐	☐	☐	☐
j.	Feeling sad or blue	☐	☐	☐	☐	☐
k.	Feeling angry	☐	☐	☐	☐	☐
l.	Trouble sleeping	☐	☐	☐	☐	☐
m.	Worrying	☐	☐	☐	☐	☐
	Social functioning (problems with…)					
n.	Playing with other children	☐	☐	☐	☐	☐
o.	Other kids not wanting to play with him or her	☐	☐	☐	☐	☐
p.	Getting teased by other children	☐	☐	☐	☐	☐
q.	Not able to do things that other children his or her age can do	☐	☐	☐	☐	☐
r.	Keeping up when playing with other children	☐	☐	☐	☐	☐
	Day-care functioning – please complete this section if your child attends day-care (problems with...)					
s.	Doing the same day-care activities as peers	☐	☐	☐	☐	☐
t.	Missing day-care because of not feeling well	☐	☐	☐	☐	☐
u.	Missing day-care to go to the doctor	☐	☐	☐	☐	☐

Raspberry

4.8 The next questions are about your child's emotional well-being and behaviour. These can be big issues for 4-year-olds. It would help us If you answered the Items as best you can even If you are not absolutely certain. Please give your answers on the basis of the child's behaviour over the last 6 months.

		Not True	Somewhat True	Certainly True
a.	considerate of other people's feelings	D	D	D
b.	restless, overactive, cannot stay still for long	D	D	D
c.	often complains of headaches, stomach-aches or sickness	D	D	D
d.	shares readily with other children, for example toys, treats, pencils	D	D	D
e.	often loses temper	D	D	D
f.	rather solitary, prefers to play alone	D	D	D
g.	generally well behaved, usually does what adults request	D	D	D
h.	many worries or often seems worried	D	D	D
i.	helpful if someone is hurt, upset or feeling ill	D	D	D
J.	constantly fidgeting or squirming	D	D	D
k.	has at least one good friend	D	D	D
l.	often fights with other children or bullies them	D	D	D
m.	often unhappy, depressed or tearful	D	D	D
n.	generally liked by other children	D	D	D
o	easily distracted, concentration wanders	D	D	D
p.	nervous or clingy in new situations, easily loses confidence	D	D	D
q.	Kind to younger children	D	D	D
r.	often lies or cheats	D	D	D
s.	picked on or bullied by other children	D	D	D
t	often volunteers to help others (parents, teachers, other children)	D	D	D
u.	thinks things out before acting	D	D	D
v.	steals from home, school or elsewhere	D	D	D
w.	gets along better with adults than with other children	D	D	D
x.	many fears, easily scared	D	D	D
y.	good attention span, sees tasks through to an end	D	D	D

4.9 At what time does your child usually go to bed at night? ☐ pm

4.1 O What is their usual wake-up time in the morning? ☐ am

5. QUESTIONS ABOUT YOUR FAMILY

5.1 Since we first saw you when your child was 1 year old, has anyone in your immediate family developed any <u>new symptoms</u> of .....

		No one	4 year old child	Child's mother	Child's father	Child's brother 1	Child's brother 2	Child's sister 1	Child's sister 2	other sibling
a.	Asthma	☐	☐	☐	☐	☐	☐	☐	☐	☐
b.	Eczema	☐	☐	☐	☐	☐	☐	☐	☐	☐
c.	Hay fever	☐	☐	☐	☐	☐	☐	☐	☐	☐
d.	Latex allergy	☐	☐	☐	☐	☐	☐	☐	☐	☐
e.	Insect allergy which insect: ☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
f.	Food allergy which foods: ☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

5.2 Do you <u>currently</u> have pets at home?

☐ No ---→ go to Question 5.3 ☐ Yes

If yes,

			Inside and outside	Outside only	Inside only
a.	Number of cats:	☐	☐	☐	☐
b.	Number of dogs:	☐	☐	☐	☐
c.	Number of birds:	☐	☐	☐	☐
d.	Other pet *(please specify)* ☐	☐	☐	☐	☐

5.3 Does your household <u>currently</u> keep your pets outside or avoid having pets at home because of allergy?

☐ No ☐ Yes, avoid cats only ☐ Yes, avoid dogs only ☐ Yes, avoid both cats and dogs

5.4 How many people in your household regularly smoke (most days of the week)? ☐

5.5 What is the total number of cigarettes smoked by all residents **<u>outside</u>** your home?

☐ per week

5.6 What is the total number of cigarettes smoked by all residents **<u>inside</u>** your home?

☐ per week

5.7 Does anyone smoke in the same room as the child?

☐ Never ☐ Sometimes ☐ Usually

Raspberry

5.8 What is the highest education or vocational qualification completed by the child's....

	a. mother	b. father
year 10 or less	☐	☐
year 11	☐	☐
year 12	☐	☐
trade apprenticeship	☐	☐
technical diploma/certificate	☐	☐
university degree	☐	☐
postgraduate university degree	☐	☐
Other *(please specify)*	☐	☐

☐

5.9 a. Is the child's **mother** currently in paid work? ☐ No ☐ Part-time ☐ Full-time

b. Is the child's **father** currently in paid work? ☐ No ☐ Part-time ☐ Full-time

Thank you for completing this questionnaire.
Please check that you've answered all the questions, on both sides of each page and filled in the date on Page 1.
Please return it to us in the reply paid envelope.
For queries, contact the HealthNuts team
on (03) 8341 6266 or health.nuts@mcri.edu.au.

We'll contact you again when your child turns 6 years old to ask about their food allergy, eczema, asthma and hay fever once they are at school. Just in case your address or phone number changes, is there a friend or relative (such as a grandparent or aunt/uncle) we could contact?

Given name(s): ☐

Surname: ☐

Relationship to the child: ☐

Address – Number and Street: ☐

Address – Suburb: ☐ Postcode: ☐☐☐☐

Phone: Mobile ☐☐☐☐ ☐☐☐ ☐☐☐ Home (☐☐) ☐☐☐☐ ☐☐☐☐

Email: ☐

Murdoch Childrens Research Institute

Appendix 3. HealthNuts Age 6 Questionnaire

Health Nuts
Nuts About Health

HealthNuts Questionnaire

HealthNuts ID ☐☐☐☐

PLEASE COMPLETE THIS SECTION BEFORE STARTING THE QUESTIONNAIRE

Thank you for helping with our research. All your information is confidential. If you have any questions or need help filling out this questionnaire, please contact the HealthNuts study team by:

- Phone (03) 8341 6266
- Email health.nuts@mcri.edu.au

Please use a black or blue pen.

CONTACT DETAILS

Child

Given name(s): Surname:

For *child's* mother (or guardian)

Given name(s): Surname:

Address – Number and Street:

Address – Suburb: Post code: State:

Phone: Mobile: Home ( )

Work ( ) Email:

For *child's* father (or guardian)

Given name(s): Surname:

Address – Number and Street:

Address – Suburb: Post code: State:

Phone: Mobile: Home ( )

Work ( ) Email:

QUESTIONS FOR THE PERSON COMPLETING THIS QUESTIONNAIRE

Today's date: ☐☐/☐☐/☐☐☐☐ Child's date of birth: ☐☐/☐☐/☐☐☐☐

Your Given name(s): Your Surname:

Are you this child's....? ☐ Biological parent ☐ Step parent ☐ Other

Are you ....? ☐ Female ☐ Male

We'll contact you shortly to organise a time for your child's allergy assessment.
Please fill in the extra details below to help with this.

Who is the best person to contact?

What is the best number to use? *(tick all that apply)* ☐ Home ☐ Mobile ☐ Work

What are the best days and times?

MCRIAV1-1

1. FEEDING YOUR CHILD

We last were in contact with you when your child was 4 years old. We want to know what new foods you've introduced into your child's diet since that time. Since age 4, has your child eaten the following foods? *(tick one box on each line)*

	Never in child's life	Yes, but not in the last 2 years	Yes, eaten 1-3 times in the last 2 years	Yes, eaten more than 3 times in the last 2 years
u Peanut butter	O,	02	03	04
1.2 Peanuts	O,	02	03	04
1.3 Pistachios	O,	02	03	04
1.4 Cashews	O,	02	03	04
1.5 Almonds	O,	02	03	04
1.6 Hazelnuts (including Nutella)	O,	02	03	04
1.7 Pine nuts	O,	02	03	04
1.8 Other nuts *(please specify)*	O,	02	03	04
1.9 Tahini (or hummus)	0,	02	03	04
1.10 Sesame seeds on foods (e.g. bread, sesame snaps)	0,	02	03	04
1.11 Semi-cooked (runny) egg (e.g. scrambled, soft boiled, fried, poached)	0,	02	03	04
1.12 Completely cooked (hard) egg (e.g. hard boiled, fried, poached)	0,	02	03	04
1.13 Meringue, pavlova or macaroons	0,	02	03	O
1.14 Cakes containing egg	0,	02	03	04
1.1 5 Biscuits containing egg (e.g. teddy bear biscuits)	0,	02	03	04
1.16 Other foods containing egg *(please specify)*	0,	02	03	04
1.17 Fish	0,	02	03	04
1.18 Shellfish *(please specify)*	0,	02	Os	D.
1.19 Soy milk or other soy products *(please specify)*	0,	02	Os	0.
1.20 Cow's milk (including on cereal)	0,	02	03	D.
1.21 Cow's milk in baked products (e.g. cakes, muffins)	0,	02	03	04
1.22 Other dairy products (e.g. cheese, yoghurt, cream, ice cream) *(please specify)*	0,	02	03	D.
1.23 Other types of milk (e.g. goat's milk) *(please specify)*	O,	□2	□3	04
1.24 Wheat (e.g. bread, cakes, biscuits)	O,	□2	□3	O.

1.25 Do you restrict any particular foods in your child's diet? O1 No O2 Yes

If yes, (a) which foods does your child avoid?

(b) why are these foods avoided?

1.26 In the past 12 months, how often, on average, did your child eat or drink the following?
{Please leave blank i fyou do not know what a food is) {tick one box on each line)

		Never or occasionally	Once or twice per week	Three or more times a week
a)	Meat (e.g. beef, lamb, chicken, pork)	0,	02	Oa
b)	Seafood (including fish)	0,	02	Oa
c)	Oily fish (e.g. salmon, fresh tuna, trout, mackerel, sardines)	0,	02	Oa
d)	Fruit	0,	02	Oa
e)	Vegetables (green and root)	0,	02	Oa
f)	Pulses (peas, beans, lentils)	0,	02	Oa
g)	Cereal (including bread)	0,	02	03
h)	Pasta	0,	02	Oa
i)	Rice	0,	02	03
j)	Butter	D,	02	Oa
k)	Margarine	0,	02	Oa
l)	Nuts	0,	02	03
m)	Potatoes	0,	02	03
n)	Cow's milk *Please specify type (e.g. full fat, low fat etc)*	0,	02	Oa
o)	Other types of milk *Please specify type (e.g. soy, almond etc)*	0,	02	03
p)	Eggs	0,	02	Oa
q)	Fast food *I* takeaway	0,	02	03
r)	Soft drink	0,	02	03
s)	Cordial	0,	02	03
t)	Fruit juice	0,	02	Oa
u)	Powdered nutritional supplements *(e.g. Sustagen)*	0,	02	Oa

1.27 Has your child ever been fed toddler formula or follow-on formula (e.g. Karicare Toddler or Heinz Nurture Follow-on)?

O, No - go to Cluestion 21

O₂ Yes - a. At what age did they start? [ ] year(s) or [ ] months

b. At what age did they stop? [ ] year(s) or [ ] months

c. What was the name/s of the formula/s?

[ ]

2. FOOD ALLERGIES AND INTOLERANCES

2.1 **Since we last were in contact with you (child age 4), has your child had a reaction which you thought was due to some food they had eaten?**

O 1 No - - - + goto Question 2.2 O 2 Yes *(answerquestionsbelow)....*

To which food(s)	How old were they?		What was the reaction? *(please tick all that apply)*							How long after food eaten was the reaction?		
	years & months		hives/ urticaria/ wheals	facial swelling	vomiting	diarrhoea	breathing problems (e.g. cough, wheeze, shortness of breath)	eczema flare	other reaction *(please specify):*	less than 1 hr	1-4 hrs	more than 4 hrs
EXAMPLE:	Years	Months										
Peanut	ID	D	O	O	O	O	O	O	lred ss on fece	O	O	O
a	100	DD	O	O	O	O	O	O		O,	02	Os
b	IDD	DD	O	O	O	O	O	O		O,	02	Os
c	IDD	DD	O	O	O	O	O	O		O,	02	Os
d	100100		O	O	O	O	O	O		O,	02	Os
e	IDD, DD		O	O	O	O	O	O		D,	02	Os
f	100	DD	O	O	O	O	O	O		01	02	Os
g	100	DD	O	O	O	O	O	O		O,	02	Os

READ THIS CAREFULLY
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2.2 Since age 4, have you consulted any health professionals about your child's food reactions?

No O ---+ If no, go to Question 31

a. Doctor - general practitioner (GP) O How many times did you see a GP? [ ]

b. Paediatrician O How many times did you see a paediatrician? [ ]

c. Allergist O How many times did you see an allergist? [ ]

d. Other health professional *(please specify)* O How many times did you see this health professional? [ ]

[ ]

e. Complementary medicine practitioner *(please specify)* O How many times did you see a complementary medicine practitioner? [ ]

[ ]

2.3 Does your child currently have an EpiPenlAnapen for food allergy? O, No O 2Yes

3. RASHES, CHEST SYMPTOMS, RUNNY NOSE

Rashes

3.1 Has your child ever had an itchy rash which was coming and going for at least six months?

O 1No ---+ goto Question 32 O 2Yes O 3Don't know

If yes...

a. Has your child had this itchy rash at any time in the last 12 months?
O 1No O 2Yes O 3Don't know

b. Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?
O 1No O 2Yes O 3Don't know

c. At what age did this itchy rash first occur?
O, Under 2 years O 2Age 2-4 years O , Age 5-6 years

d. Has this rash cleared completely at any time in the last 12 months?
O 1No O 2Yes O 3Don't know

e. In the last 12 months, how often, on average, has your child been kept awake at night by this itchy rash?
O 1 Never in the last 12 months
O 2 Less than one night per week
O 3 One or more nights per week

3.2 Has your child been diagnosed with eczema?
O 1 No O2 Yes --+ a. Age when symptoms started D year(s) or D months old

3.3 In the last 12 months, has your child suffered from dry skin in general?

O, No O 2 Yes

3.4 Have you ever used medication to treat your child's eczema, itchy rash or dry skin?
(You can tick more than one box if necessary)

O Never had eczema, itchy rash or dry skin ---+ go to Question 3.5
O Had/have eczema, itchy rash or dry skin but never used ---+ go to Question 3.5
O Used in the past
O Use now

If you have ever used medication for your child's eczema, itchy rash or dry skin

a. Have you used moisturisers?
O, No O 2 Yes Name [ ] O 3 Don't know

b. Have you used topical steroid creams or ointments (e.g. sigmacort, celestone, elocon, cortic, hydrocortisone, advantan fatty ointment)?
O, No O 2 Yes Name [ ] D s Don't know

c. Did you use steroid creams for more than 10 days in a row?
O, No O 2 Yes Os Don't know

3.5 In the past 12 MONTHS, how many days (or part days) of school has your child missed because of an itchy skin rash or eczema?

O, None
O2 1 to 5
Os 6 to 10
O. More than 10

3.6 Since age 4, have you consulted any health professionals about your child's eczema or dry skin?

No O ---+ If no, go to Question 3.7

a. Doctor - general practitioner (GP) O How many times did you see a GP? [ ]
b. Paediatrician O How many times did you see a paediatrician? [ ]
c. Allergist O How many times did you see an allergist? [ ]
d. Dermatologist O How many times did you see a dermatologist? [ ]
e. Other health professional *(please specify)* [ ] O How many times did you see this health professional? [ ]
f. Complementary medicine practitioner *(please specify)* [ ] O How many times did you see a complementary medicine practitioner? [ ]

Wheezing and coughing

3.7 Has your child ever had wheezing or whistling in the chest at any time in the past?

0 1 No --- goto Question 3.8 0 2 Yes 0 3 Don't know

If yes...

a. At what age did the symptoms first start? __ year(s) old

b. Has your child had wheezing or whistling in the chest in the last 12 months?

0 1 No --- goto Question 3.8 0 2 Yes

c. How many attacks of wheezing has your child had in the last 12 months?

0 None 0 2 1 to 3 0 3 4 to 12 0 More than 12

d. In the last 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?

0 1 Never woken with wheezing
0 2 Less than one night per week
0 3 One or more nights per week

e. In the last 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths?

0 1 No 0 2 Yes

3.8 Has your child ever had asthma?

0 No
0 2 Yes --- a) Were you told by a doctor that your child had asthma? 0 1 No 0 2 Yes

b) Age when symptoms started __ year(s) old

c) Do you have a written asthma action plan which tells you how to look after your child's asthma?

0 No 0 2 Yes 0 3 Don't know

3.9 In the last 12 months, has your child used any medicines, pills, puffers or other medications for wheezing or asthma?

0 1 No --- go to Question 3.10 0 2 Yes 0 3 Not sure

If yes, please list the medications and when they were used.

	When wheezy	Regularly (every day for at least 2 months)
	(tick one box for each line)	
a. Name of 'Western' medicine		
	0	2
	0	2
	0	2
b. Name of 'Alternative' medicine		
	0	02
	0	02

3.1 O Since age 4, have you consulted any health professionals about your child's wheezing or asthma?

No O ---+ If no, go to Question 311

a. Doctor - general practitioner (GP) O How many times did you see a GP? [ ]

b. Paediatrician O How many times did you see a paediatrician? [ ]

c. Allergist O How many times did you see an allergist? [ ]

d. Other health professional *(please specify)* [ ] O How many times did you see this health professional? [ ]

e. Complementary medicine practitioner *(please specify)* [ ] D How many times did you see a complementary medicine practitioner? [ ]

3.11 In the past **12** MONTHS, how many times has your child been admitted to the hospital because of wheezing or asthma?

O 1 None
O2 1
O a 2
O. S to 5
Os 6 to 10
Os More than 10

3.12 In the past **12** MONTHS, how many days (or part days) of school has your child missed because of wheezing or asthma?

O 1 None
O 2 1 to 5
O a 6 to 10
O. More than 10

Hay fever

All questions are about problems which occur when your child DOES NOT have a cold or the flu.

3.13 Has your child ever had sneezing or a runny or blocked nose, when he/she did not have a cold or the flu?

O 1 No---+ goto Question 314 O 2 Yes O a Don't know

If yes...

a. At what age did the symptoms first start? [ ] year(s) old

b. In the past 12 months, has your child had a problem with sneezing or a runny or blocked nose when he/she DID NOT have a cold or the flu?

O 1 No ----. go to Question 314 O 2 Yes O 3 Don't know

c. In the past 12 months, has this problem been accompanied by itchy-watery eyes?

O 1 No O 2 Yes O a Don't know

d. In the past 12 months, how much did this nose problem interfere with your child's activities?

O 1 Not at all O 2 A little O 3 A moderate amount O 4 A lot

e. In which of the past 12 months did nose problems occur? *(tick all that apply)*

O January O April D July O October
O February O May D August O November
O March D June O September O December

3.14 Has your child ever had hay fever?

0 1 No ---+ go to Question 3.16
0 2 Yes ---+ a. Were you told by a doctor that your child had hay fever? 0 1 No 02 Yes
b. Age when symptoms started I___I year(s) old

3.15 Since age 4 have you consulted any health professionals about your child's hay fever?

No 0 ---+ If no, go to Question 3.16

a. Doctor - general practitioner (GP) 0 How many times did you see a GP? [ ]
b. Paediatrician 0 How many times did you see a paediatrician? [ ]
c. Allergist 0 How many times did you see an allergist? [ ]
d. Other health professional *(please specify)* 0 How many times did you see this health professional?
[ ] [ ]
e. Complementary medicine practitioner *(please specify)* 0 How many times did you see a complementary medicine practitioner?
[ ] [ ]

3.16 In the past **12** MONTHS, how much did any nose problem interfere with your child's daily activities?

0 1 Not at all
0 2 A little
0 3 A moderate amount
0. A lot

3.17 In the past **12** MONTHS, has your child used any medicines, pills, nose sprays or other medication for hay fever or nose problems?

0, No 0 2 Yes 0 3 Don't know

3.18 In the past **12** MONTHS, how many days (or part days) of school has your child missed because of hay fever or nose problems?

0 1 None
0 2 1 to 5
0 3 6 to 10
0. More than 10

'1. OTHER QUESTIONS ABOUT YOUR CHILD

4.1 In general, would you say your child's health is:

0, Excellent 0 2 Very good ☐3 Good 0. Fair O s Poor

4.2 Has your child ever had a gastrointestinal worm infection (e.g. Threadworms or Pinworms)?

0 1 No --+ go to Question 43
0 2 Yes ---+ a. How old was he/she when they first had worms?
D1 Less than 1 year 0 2 1 to 3 years 0 3 4 to 6 years
b. How many times has he/she had worms? I___.

4.3 During the last year, how much time did your child spend in the sun?

		<1 hr a day	1 to 2 hrs per day	2 to 3 hrs per day	3 to 4 hrs per day	?4 hrs a day
	Summer					
a.	on weekdays	0,	0,	03	04	Os
b.	on weekends	0,	D,	03	04	Os
	Winter					
c.	on weekdays	0,	0,	03	D.	Os
d.	on weekends	0,	0,	03	0.	Os

4.4 a. What was your child's last measured weight? DD.D kg

b. Date recorded DD/DD/DD:

4.5 a. What was your child's last measured height? DOD cm

b. Date recorded DD/DD/DD

4.6 Does your child have any brothers or sisters (including half siblings)?

0, No --- go to Ouestion 4.7 0 2 Yes - *Please provide details about the child's siblings, starting from oldest to youngest*

Number	Date of birth	Relation	Sibling type	Do they live more than % the time in the child's household?
1	DD/DD/DD	D, Sister 0, Brother	0, Full D, Half mother common	03 Half father common D. Other	0, No D, Yes
2	DD/DD/DD	D, Sister 0, Brother	D, Full 0, Half mother common	D3 Half father common D. Other	0, No 02 Yes
3	DD/DD/DD	0, Sister 0, Brother	0, Full 0, Half mother common	03 Half father common 0. Other	0, No 0, Yes
4	DD/DD/DD	0, Sister 0, Brother	0, Full 0, Half mother common	03 Half father common D. Other	0, No 02 Yes
5	DD/DD/DD	B: Sister Brother	D, Full 02 Half mother common	03 Half father common O. Other	0, No 02 Yes
6	DD/DD/DD§:	Sister Brother	0, Full 0, Half mother common	03 Half father common O. Other	01 No 02 Yes

Please list any other siblings below:

4.7 Below is a list of things that might be a problem for your child. Please tell us how much of a problem each one has been for your child in the past ONE month.

		Never	Almost Never	Some-times	Often	Almost Always
	Physical Functioning (problems with ...)					
a.	Walking more than one block	01	02	03	0.	05
b.	Running	01	02	03	O.	05
c.	Participating in sports activity or exercise	01	0,	03	O.	05
d.	Lifting something heavy	01	0,	03	0.	05
e.	Taking a bath or shower by him or herself	01	0,	03	D.	Os
f.	Doing chores, like picking up his or her toys	01	0,	03	0.	05
g.	Having hurts or aches	01	0,	03	O.	Os
h.	Low energy level	01	0,	Oa	0.	Os
	Emotional Functioning (problems with ...)					
i.	Feeling afraid or scared	01	0,	Oa	0.	Ds
j.	Feeling sad or blue	01	02	03	0.	Os
k.	Feeling angry	01	02	03	O.	Os
l.	Trouble sleeping	01	0,	03	0.	Os
m.	Worrying about what will happen to him or her	01	0,	03	0.	Os
	Social Functioning (problems with ...)					
n.	Getting along with other children	01	0,	03	0.	Os
o.	Other kids not wanting to be his or her friend	01	0,	03	O.	Os
p.	Getting teased by other children	01	0,	03	0.	Os
q.	Not able to do things that other children his or her age can do	01	0,	03	0.	05
r.	Keeping up when playing with other children	01	02	03	O.	Os
	School Functioning (problems with ...)					
s.	Pay attention in class	01	0,	03	O.	Os
t.	Forgetting things	01	0,	Oa	0.	Os
u.	Keeping up with school activities	01	0,	Oa	O.	Os
v.	Missing school because of not feeling well	0,	0,	Oa	0.	05
w.	Missing school to go to doctor or hospital	0,	0,	Oa	O.	Os

4.8 The next questions are about your child's emotional well-being and behaviour. These can be big issues for 6-year-olds. It would help us if you answered the items as best you can even if you are not absolutely certain. Please give your answers on the basis of the child's behaviour over the last 6 months.

		Not True	Somewhat True	Certainly True
a.	considerate of other people's feelings	0.	02	03
b.	restless, overactive, cannot stay still for long	D,	02	03
c.	often complains of headaches, stomach-aches or sickness	0,	02	03
d.	shares readily with other children, for example toys, treats, pencils	0,	02	03
e.	often loses temper	0,	02	03
f.	rather solitary, prefers to play alone	0,	02	03
g.	generally well behaved, usually does what adults request	D,	02	03
h.	many worries or often seems worried	0,	02	03
i.	helpful if someone is hurt, upset or feeling ill	0,	02	03
j.	constantly fidgeting or squirming	0,	02	03
k.	has at least one good friend	0,	02	03
l.	often fights with other children or bullies them	0,	02	03
m.	often unhappy, depressed or tearful	0,	02	03
n.	generally liked by other children	0,	02	03
o.	easily distracted, concentration wanders	0,	02	03
p.	nervous or clingy in new situations, easily loses confidence	0,	02	03
q.	kind to younger children	0,	02	03
r.	often lies or cheats	0,	02	03
s.	picked on or bullied by other children	0,	02	03
t.	often volunteers to help others (parents, teachers, other children)	0,	02	03
u.	thinks things out before acting	0,	02	03
v.	steals from home, school or elsewhere	0,	02	03
w.	gets along better with adults than with other children	0,	02	03
x.	many fears, easily scared	0,	02	03
y.	good attention span, sees tasks through to the end	0,	02	03

4.9 At what time does your child usually go to bed at night? ☐ pm

4.10 What is their usual wake-up time in the morning? ☐ am

5. QUESTIONS ABOUT YOUR FAMILY

5.1 Since we last were in contact with you when your child was 4 years, has anyone in your immediate family developed any new symptoms or diagnoses of .....

		Noone	Child's mother	Child's father	Child's brother 1	Child's brother 2	Child's sister 1	Child's sister 2	Other sibling
a.	Asthma	0	0	0	0	0	0	0	0
b.	Eczema	0	0	0	0	0	0	0	0
c.	Hay fever	0	0	0	0	0	0	0	0
d.	Latex allergy	0	0	0	0	0	0	0	D
e.	Insect allergy which insect:	0	0	0	0	0	0	0	0
f.	Food allergy which foods:	0	0	0	0	0	0	0	0

5.2 Do you currently have pets at home?

0, No ---.. go to Question 54 02 Yes

If yes,

			Inside and outside	Outside only	Inside only
a.	Number of cats:		0,	02	03
b.	Number of dogs:		0,	02	03
c.	Number of birds:		0,	02	03
d.	Other pet *(please specify)*		0,	02	03

5.3 Does your household currently keep your pets outside or avoid having pets at home because of allergy?

O, No O2Yes, avoid cats only O3Yes, avoid dogs only O. Yes, avoid both cats and dogs

O5 Yes, avoids other pets *(please specify)*

5.4 Do you live on a farm or property with any animals (livestock)? O1 No 02Yes

5.5 How many people in your household regularly smoke (most days of the week)?

5.6 What is the total number of cigarettes smoked by all residents outside your home?

[-- \ ] per week

5.7 What is the total number of cigarettes smoked by all residents inside your home?

[-- ',] per week

5.8 Does anyone smoke in the same room as the child?

O 1 Never O 2 Sometimes O 3 Usually

5.9 What is the highest education or vocational qualification completed by the child's....

	a. mother	b. father
year 10 or less	O,	☐ 2
year 11	O1	☐ 2
year 12	O,	☐ 2
trade apprenticeship	O,	☐ 2
technical diploma/certificate	O,	☐ 2
university degree	O,	☐ 2
postgraduate university degree	O,	☐ 2
Other *(please specify)*	O,	☐ 2

[ ]

5.10 a. Is the child's mother currently in paid work? O 1 No O 2 Part-time O a Full-time

b. Is the child's father currently in paid work? O 1 No O 2 Part-time O 3 Full-time

5.11 a. Child's mother's current weight
(if currently or recently pregnant, record pre-pregnancy weight): DDD.D kg

b. Child's mother's height: DDD cm

c. Child's mother's date of birth: DDIDDIDD

5.12 a. Child's father's current weight: DDD.D kg

b. Child's father's height: DDD cm

c. Child's father's date of birth: DDIDDIDD

5.13 In which country/region was your child born?

O, Australia / New Zealand
O 2 Europe
O 3 India / Pakistan / Bangladesh
O 4 Africa
O s United Kingdom
O s America
D 7 Asia (e.g. Singapore, Malaysia, Vietnam)
O 8 Middle East (e.g. Egypt, Syria)
O s Other [ ]

5.14 Child's mother's ethnicity?

- O 1 Caucasian
- O 2 Asian
- O a African
- O 4 Aboriginal or Torres Strait Islander
- O s Middle Eastern
- O 6 Other *(please specify)*

5.15 Child's father's ethnicity?

- 0 1 Caucasian
- 0 2 Asian
- O a African
- 0 4 Aboriginal or Torres Strait Islander
- O s Middle Eastern
- O 6 Other *(please specify)*

5.16 What is the ethnicity of your child's natural GRANDPARENTS? (i.e., their ethnic origin)

Mother's Parents:		Father's Parents:	
Mother *(tick one box)*	Father *(tick one box)*	Mother *(tick one box)*	Father *(tick one box)*
0, Caucasian	0, Caucasian	0, Caucasian	0, Caucasian
02 Asian	02 Asian	02 Asian	L·02 Asian
0a African	0a African	0a African	03 African
04 Aboriginal or Torres Strait Islander	04 Aboriginal or Torres Strait Islander	0. Aboriginal or Torres Strait Islander	0. Aboriginal or Torres Strait Islander
0s Middle Eastern	0s Middle Eastern	0s Middle Eastern	0s Middle Eastern
0s Other *(please specify)*	0s Other *(please specify)*	0s Other *(please specify)*	0s Other *(please specify)*

READ THIS CAREFULLY

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Changes required:_____

Ok to print:_____

Date:_____

Thank you for completing this questionnaire.
Please check that you've answered all the questions, on both sides of each page and filled in the date on Page 1.
Please return it to us in the reply paid envelope.
For queries, contact the HealthNuts team on (03) 8341 6266 or health.nuts@mcri.edu.au

Just in case your address or phone number changes, is there a friend or relative (such as a grandparent or aunUuncle) we could contact?

Given name(s):

Surname:

Relationship to the child:

Address - Number and Street:

Address - Suburb: Postcode: DD DD State: DD D

Phone: Mobile DODD ODD ODD Home (DD)DDDD DODD

Email:

Appendix 4. HealthNuts Age 1 Non-Responder Questionnaire

NON-RESPONDER QUESTIONNAIRE

Circle the relevant answer for potential participants that decline to participate:

- history of eczema, asthma and hay fever in mother

- history of wheeze and rash in child

- previous child with asthma or allergy

sex,

age (months)

area of residence (via postcode) _ _ _ _

Appendix 5. SchoolNuts Student Questionnaire

Murdoch Childrens Research Institute
Healthier Kids. Healthier Future.

School Nuts
Nuts About School

SN ID ☐☐☐☐☐

SchoolNuts student questionnaire – secondary

This questionnaire is about your health and well being and in particular your thoughts, feelings and attitudes towards food allergy and asthma.

There are no right or wrong answers.

For most questions there is a choice of answers.
Please tick ONE box only unless otherwise requested.

Pick the one that's most true for you and tick the box next to it like this:

No ☐ Yes ☑

If you make a mistake, simply scribble it out and mark the correct answer with a tick like this:

No ■ Yes ☑

All information is private and confidential.

If you have a question please ask one of the researchers for help.

Let's start!

Your name: _____

Name of your school: _____

The Royal Children's Hospital Melbourne

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QUESTIONS ABOUT YOU

1 What is your date of birth?

DD/DD/DODD

2 Are you?

0 1 Male 0 2 Female

3 How old are you?

DD yearsold

4 What year are you in?

Years	01
Year6	0 2
Year 7	03
Year8	04

5 What class are you in? i.e. 5a, 5b, 5c, 5d etc

6 In what country were you born?

Australia ☐ 1

Other *(please specify)* ☐ 2

WHAT DO YOU KNOW ABOUT FOOD ALLERGY?

The next questions ask what you know about food allergy.

		True	False	Don't know
7.	A person can die from having a food allergy reaction	01	02	03
8.	Red bumps on the skin that can be itchy (called 'hives') are a common symptom of a food allergy reaction	0,	02	03
9.	People with a food allergy can die after touching a food	0,	02	03
10.	Food allergies run in families	01	02	03
11.	Food allergies can go away as a person gets older	0,	02	03
12.	The number of children with a food alle gy in Australia has been increasing over the past 10 years	01	02	03
13.	There is a cure for food allergy	0,	02	03
14.	The only way to prevent a food allergy reaction is to stay away from the food that causes the allergy	01	02	03

15 **A boy with a milk allergy accidently drank some milk and had an allergic reaction. There are 4 statements which give possible symptoms to the reaction. For ALL 4 statements please answer true/false or don't know**

		True	False	Don't know
a)	After 2 days he gets a headache	01	02	03
b)	After 10 minutes he has hives on his face and chest	0,	02	03
c)	Immediately his tongue swells and he has trouble breathing	0,	02	03
d)	He has a stuffy nose that won't go away for weeks	0,	02	03

16 **Which are the three most common food allergies in children?**
(please tick 3 answers)

Egg	0
Broccoli	0
Peanut	0
Kiwi-fruit	0
Shellfish (eg. prawns, lobster, crab)	0
Tree nuts (eg. almonds, walnuts, hazelnuts, cashews)	0
Milk	D
I don't know	0

17 **Which age group is most likely to have food allergies?**

0-5 years	☐ 1
6-10 years	☐ 2
11 years or older	☐ 3
I don't know	☐ 4

WHAT DO YOU THINK ABOUT FOOD ALLERGY?

The next questions ask what you think about food allergy.

		Strongly disagree	Disagree	Neither	Agree	Strongly agree
18.	People with food allergies are treated differently because of their food allergy	01	02	03	04	Ds
19.	Children with food allergy have over-protective parents	01	02	03	04	Ds
20.	Children with food allergies are teased at school	Di	02	03	04	Ds
21.	Someone with a food allergy has trouble staying away from the food they are allergic to	D,	02	03	04	Ds
22.	People with food allergies worry a lat about their food allergy	01	02	03	04	Ds
23.	It is difficult for people with food allergies to safely eat at a restaurant	01	02	03	04	Ds
24.	Having an EpiPen or AnaPen is important for most children with food allergies	01	02	03	04	Ds
25.	Schools should have plans for children with food allergies	D,	02	03	04	Ds
26.	Schools should ban all foods with nuts	D,	02	03	04	Ds
27.	Schools should have special tables where children with food allergies can eat lunch	D	02	03	04	Ds
28.	It would be unfair if I could not eat something at school because of another student's food allergy	D.	02	03	04	Ds

ABOUT YOUR HEALTH IN GENERAL

The next questions are about things that might be a problem for you.

In the past one month, how much of a problem has this been for you?

About My Health and Activities (Problems with...)		Never	Almost never	Sometimes	Often	Almost always
29.	It is hard for me to walk more than one block	D,	02	03	04	Ds
30.	It is hard for me to run	D.	02	03	04	Ds
31.	It is hard for me to do sports activity or exercise	D.	02	03	04	Ds
32.	It is hard for me to lift something heavy	D1	02	03	04	Ds
33.	It is hard for me to take a bath or shower by myself	D,	02	03	04	Ds
34.	It is hard for me to do chores around the house	01	02	03	04	Ds
35.	I hurt or ache	D,	02	03	04	Ds
36.	I have low energy	D.	02	03	04	D!i

About M Feelings {n_roblems with...l	Never	Almost never	Sometimes	Often	Almost always
37. I feel afraid or scared	01	02	03	04	Ds
38. I feel sad or blue	D,	02	03	04	Os
39. I feel angry	D,	02	03	04	Os
40. I have trouble sleeping	01	02	Oa	04	Ds
41. I worry about what will happen to me	D,	02	Oa	04	Os

How I Get Along With Others {n_roblems with...l	Never	Almost never	Sometimes	Often	Almost always
42. I have trouble getting along with other teens	D,	02	Oa	04	Ds
43. Other teens do not want to be my friend	D,	02	Oa	04	Os
44. Other teens tease me	D,	02	03	04	Ds
45. I cannot do things that other teens my age can do	01	02	Oa	04	Ds
46. It's hard to keep up with my peers	D,	02	Oa	04	Ds

About School {n_roblems with...l	Never	Almost never	Sometimes	Often	Almost always
47. It is hard to pay attention in class	D,	02	03	04	Os
48. I forget things	01	02	03	04	Ds
49. I have trouble keeping up with my schoolwork	D,	02	03	04	Ds
50. I miss school because of not feeling well	01	02	Oa	04	Ds
51. I miss school to go to the doctor or hospital	01	02	03	04	Ds

YOUR FEELINGS

The next questions ask about your feelings. You may or may not have these feelings. Remember all your answers will remain private. Please put a tick in the box that shows how often each of these things happen to you.

	Never	Sometimes	Often	Always
52. I worry about things	D,	02	03	D
53. I am scared of the dark	01	02	Oa	04
54. When I have a problem, I get a funny feeling in my stomach	01	02	03	04
55. I feel afraid	01	02	Oa	04
56. I would feel afraid of being on my own at home	D,	02	03	04
57. I feel scared when I have to take a test	01	02	03	04

	Never	Sometimes	Often	Always
58. I feel afraid if I have to use public toilets or bathrooms	01	02	03	04
59. I worry about being away from my parents	0,	02	03	04
60. I feel afraid that I will make a fool of myself in front of people	O1	02	03	04
61. I worry that I will do badly at my school work	O..	02	03	04
62. I am popular amongst other kids my own age	01	02	03	04
63. I worry that something awful will happen to someone in my family	01	02	03	04
64. I suddenly feel as if I can't breathe when there is no reason for this	01	02	03	04
65. I have to keep checking that I have done things right (like the switch is off, or the door is locked)	01	02	03	04
66. I feel scared if I have to sleep on my own	01	02	03	04
67. I have trouble going to school in the mornings because I feel nervous or afraid	0,	02	03	04
68. I am good at sports	O.	02	03	04
69. I am scared of dogs	0.	02	03	04
70. I can't seem to get bad or silly thoughts out of my head	0,	02	03	04

	Never	Sometimes	Often	Always
71. When I have a problem, my heart beats really fast	0,	02	03	04
72. I suddenly start to tremble or shake when there is no reason for this	01	02	03	04
73. I worry that something bad will happen to me	0,	02	03	04
74. I am scared of going to the doctors or dentists	01	02	03	04
75. When I have a problem, I feel shaky	0,	02	03	04
76. I am scared of being in high places or lifts (elevators)	01	02	03	04
77. I am a good person	0,	02	03	04
78. I have to think of special thoughts to stop bad things from happening (like numbers or words)	01	02	03	04
79. I feel scared if I have to travel in the car, or on a bus or a train	0,	02	03	04
80. I worry what other people think of me	0,	02	03	04
81. I am afraid of being in crowded places (like shopping centre's, the movies, buses, busy playgrounds)	0,	02	03	04
82. I feel happy	0,	02	03	04
83. All of a sudden I feel really scared for no reason at all	0,	02	03	04
84. I am scared of insects or spiders	0,	02	03	04
85. I suddenly become dizzy or faint when there is no reason for this	O.	02	03	04

		Never	Sometimes	Often	Always
86.	I feel afraid if I have to talk in front of my class	01	02	03	04
87.	My heart suddenly starts to beat too quickly for no reason	01	02	03	04
88.	I worry that I will suddenly get a scared feeling when there is nothing to be afraid of	01	02	03	04
89.	I like myself	01	02	03	04
90.	I am afraid of being in small closed places, like tunnels or small rooms	01	02	03	04
91.	I have to do some things over and over again (like washing my hands, cleaning or putting things in a certain order)	01	02	03	04
92.	I get bothered by bad or silly thoughts or pictures in my mind	01	02	03	04
93.	I have to do some things in just the right way to stop bad things happening	01	02	03	04
94.	I am proud of my school work	01	02	03	04
95.	I would feel scared if I had to stay away from home overnight	01	02	03	04

96. Is there something else that you are really afraid of? 01 Yes 02 No

a. Please write down what it is _____

b. How often are you afraid of this thing? 01 02 03 04

QUESTIONS FOR EVERYONE TO ANSWER:

This is to find out if you have ever had asthma or food allergy/reaction.
There will be more detailed questions later if you have had either or both.

97.	Have you EVER had a food allergy?	01 No	02 Yes
98.	Do you CURRENTLY have a food allergy?	01 No	02 Yes
99.	Have you EVER had an allergic reaction to a food?	01 No	02 Yes
100.	Have you EVER had anaphylaxis to a food?	01 No	02 Yes
101.	Have you EVER had asthma?	01 No	02 Yes
102.	Do you STILL have asthma?	01 No	02 Yes

If you CURRENTLY or STILL have:

FOOD ALLERGY ..... please go to question 103 (page 9)

ASTHMA but no food allergy ..... please go to question 144 (page 15)

If you DON'T HAVE either food allergy or asthma you have reached the end of the questionnaire. Thank you for participating

ABOUT YOUR FOOD ALLERGY

If you *DO* NOThave a food allergy please DO NOT complete these questions.

103 Please tell us which food(s) you are allergic to ***(you can tick all that apply):***

a. Peanut D
b. Other nuts *(specify):* D

c. Hen's egg D
e. Cow's milk D
f. Sesame D
g. Fish D
h. Shellfish D
i. Soy D
j. Other food *(specify):* D

104 At school who knows you have a food allergy? ***(Please tick ALL that apply)***

No one D
School staff/my teacher D
My best friends D
My friends in my class D
Every one in my class D
Friends at school D
Every one at school D
Other *(please specify)* D

105 Have you had an allergic reaction to a food in the past 12 months? ***(Please tick one)***

No D_1 .... If no please go to question 107 (page 11)
Yes D_2 .... if yes please go to question 106 (page 10)

106 Please complete this table *ONLY* if you have had a food reaction in the past 12 months.

What reactions to any food have you had in the last 12 months.				What was the reaction? *(Please tick* **ALL** *the boxes that apply)*						How long after you ate the food was the reaction?			
Please list ALL foods *(you may need to list some foods twice if you have reacted to that food more than one time)* **EXAMPLE: 1 Peanut, 2 Peanut, 3 Walnut**	Was this reaction anaphylaxis? *(Please tick)*	Was an EpiPen or Anapen used on you? *(Please tick)*	Where did the reaction happen?	Skin rash	Facial swelling	Vomiting	Diarrhoea	Breathing difficulties (eg: cough, wheeze)	Other reaction *(please specify):*	less than 1 hr	1-4 hrs	more than 4 hrs	don't know
1.1	01 Yes 02 No 03 Don't know	01 Yes 02 No 0a Don't know	01 School 02 Home 03 Friend's house 04 Restaurant 0s Other	0	0	D	0	D	D	01	02	0a	04
2.1	01 Yes 02 No 0a Don't know	01 Yes 02 No 03 Don't know	01 School 02 Home 03 Friend's house 04 Restaurant 05 Other	D	0	0	0	D	0	01	02	03	04
3.1	01 Yes 02 No 03 Don't know	01 Yes 02 No 0a D n't know	01 School 02 Home 03 Friend's house 04 Restaurant 0s Other	0	D	0	0	0	D	01	02	03	04
4.1	01 Yes 02 No 0s Don't know	0, Yes 02 No 03 Don't know	01 School 02 Home 03 Friend's house 04 Restaurant 05 Other	0	0	0	D	0	0	01	02	03	04

107 Do you CURRENTLY have an EpiPen/AnaPen?

No 0, If no please go to question 110

Yes 02

108 Do you carry your Epipen/AnaPen with you?

	Always	Sometimes	Never
While at school?	0,	02	Os
While on other school activities? i.e. drama, excursions, school dance	0,	02	Os
While on school camp?	D,	02	Os
In your sports bag when playing sport?	D,	02	Os
While at a friend's house?	0,	02	03
When out with friends?	0,	02	Os
When out by yourself?	0,	02	03
While out at a restaurant?	01	02	03
When out with your family?	0,	02	03

109 Do you have an EpiPen/Anapen at school? 01 No 02 Yes

If yes:

a) How many? [ ]

b) Where is/are your EpiPen/Anapen kept? *(Tick all that apply)*

Central office	0	With teacher	0
Your locker	0	Class room	0
Your schoolbag	0	Don't know	0
Carried with you	0	Other location	0

	Never	Sometimes	Often	Always
110. Have you EVER deliberately eaten food to which you knew you were allergic?	0,	02	Os	04
111. Have you EVER deliberately eaten food which was labelled "may contain traces of" a food you knew you were allergic to?	D,	02	03	04
112. Have you ever been teased or bullied because of your food allergy?	0,	02	03	04

HOW DOES'.FOQD ALLERGY AFFECJ" YOUB LIFE?

The next questions ask how food allergy affects your life.

How ***troublesome*** do you find it, because of your food allergy, you...							
113. must always be alert as to what you are eating?	D 0	01	02	03	04	Ds	Ds
114. are able to eat fewer products?	Do	D,	02	03	04	Ds	Ds
115. are limited as to the products you can buy?	Do	D,	02	03	04	Ds	Ds
116. must read labels?	Do	D,	02	03	04	Ds	Ds
117. have the feeling that you have less control.of what you eat when eating out?	Do	D,	02	03	04	Ds	Ds
118. are less able to spontaneously accept an invitation to stay for a meal?	Do	01	02	03	04	Ds	Da
119. are less able to taste or try various products when eating out?	Do	D,	02	03	04	Ds	Ds
120. must check yourself whether you can eat something when eating out?	Do	D,	02	03	04	Ds	Ds
121. have to check yourself whether you can eat something when eating out?	Do	D,	02	03	04	Ds	De
122. hesitate eating a product when you have doubts about it?	Do	01	02	03	04	Ds	Ds
123. must refuse treats at school or work?	Do	01	02	03	04	Ds	Ds
124. must be careful about touching certain foods?	Do	D,	02	03	04	Ds	Ds
125. must carry an EpiPen/AnaPen?	Do	D,	02	03	04	Os	Os
126. if you don't have an EpiPen/AnaPen please tick here D							

How ***troublesome*** is it, because of your food allergy...							
127. that the ingredients of a product change?	Do	D,	02	03	04	Os	De
128. that the label states: 'May contain traces of . . .'?	Do	D,	02	03	04	Os	Os
129. that the labeling of bulk packaging (for example box or bag) is different than the individual packages?	Do	D,	02	03	04	Ds	Ds
130. that you have to explain to people around you that you have a food allergy?	Do	D,	02	03	04	Os	Ds
131. that during social activities others can eat the food to which you are allergic?	Do	0,	02	03	04	Ds	Ds
132. that during social activities your food allergy is not taken into account enough?	Do	0,	D2	03	04	Ds	De

How ***frightened*** are you because of your food allergy ...

133 of an allergic reaction?	Do	D,	02	Da	04	05	Ds
134. of accidently eating something wrong?	Do	01	02	Da	04	05	Ds
135. to eat something you have never eaten before?	Do	01	02	Da	04	05	Ds

Please answer the following questions:

136. How *discouraged* do you feel during an allergic reaction?	Do	01	02	Da	04	05	Ds
137. How *disappointed* are you when people don't take your food allergy into account?	Do	01	02	Da	04	05	Ds

The next questions ask about the chance you think something will happen to you because of your food allergy. Choose one of the following answers:

0	1	2	**3**	**4**	5	**6**
never (0% chance)	very small chance	small chance	fair chance	great chance	very great chance	always (100% chance)

How great do you think the chance is that you ...

	0 never	1	**2**	**3**	**4**	5	**6** always
138. will accidently eat something to which you are allergic?	Do	D,	02	Da	04	Ds	Ds
139. will have a severe reaction if you accidently eat something to which you are allergic?	Do	D,	02	Da	04	05	Ds
140. will die if you accidently eat something to which you are allergic?	Do	D.	02	•03	04	Ds	Ds
141 .can not effectively deal with an allergic reaction should you accidently eat something to which you are allergic?	Do	D,	02	Da	04	05	Ds

142 **How many products must you avoid because of your food allergy?**

almost none	01
very few	02
a few	03
some	04
many	Os
very many	06
almost all	07

143 **How great is the impact of your food allergy on your social life?**

negligibly small	01
very small	02
small	03
moderate	04
great	Os
very great	06
extremely great	07

If you:

HAVE asthma go to question 144 (page 15 - next page).

„ DON'T HAVE asthma you have reached the end of the questionnaire

Thank you for participating •

ABOUT YOUR ASTHMA

If you ***DO NOT*** have asthma please DO NOT complete these questions.

HOW HAS ASTHMA AFFECTED YOU

The next questions ask about how you have been affected by *asthma during the past week.*

144 **On average, during the past week, how often were you woken by your asthma during the night?**

Never	01
Hardly ever	D2
A few minutes	03
Several times	04
Many times	D0
A great many times	Ds
Unable to sleep because of asthma	D,

145 **On average, during the past week, how bad were your asthma symptoms when you woke up in the morning?**

No symptoms	01
Very mild symptoms	02
Mild symptoms	03
Moderate symptoms	04
Quite severe symptoms	06
Severe symptoms	De
Very severe symptoms	D,

"**146** **In general, during the past week, how limited were you in yotlr activities because of your asthma?**

Not limited at all	☐1
Very slightly limited	☐2
Slightly limited	☐3
Moderately limited	☐4
Very limited	☐5
Extremely limited	☐6
Totally limited	☐7

147 **In general, <u>during the past week,</u> how much shortness of breath did you experience because of your asthma?**

None	D,
A very little	02
A little	Oa
A moderate amount	04
Quite a lot	05
A great deal	06
A very great deal	07

148 **In general, <u>during the past week,</u> how much of the time did you wheeze?**

Not at all	☐ 1
Hardly any of the time	☐ 2
A little of the time	☐ 3
A moderate amount of the time	☐ 4
A lot of the time	☐ 5
Most of the time	☐ 6
All the time	☐ 7

149 **On average, <u>during the past week,</u> how many puffs of short-acting bronchodilator (e.g. Ventolin) have you used each day?**

None	☐ 1
1-2 puffs most days	☐ 2
3-4 puffs most days	☐ 3
5-8 puffs most days	☐ 4
9-12 puffs most days	☐ 5
" 13-16 puffs most days	☐ 6
More than 16 puffs most days	☐ 7

You have reached the end of the questionnaire! Thank you for participating

Appendix 6. SchoolNuts Parent Questionnaire

PARENT QUESTIONNAIRE

SchoolNuts: food allergy, asthma and risk of anaphylaxis in school-aged children and adolescents

We would like everyone to **complete** this questionnaire

Even if your child does not have a food allergy or asthma, your answers will help us understand why the number of young people with these conditions is increasing.

There are no right or wrong answers. For most questions there is a choice of answers.

Please cross ONE box only unless otherwise requested.

Pick the one that's true for you and cross the box next to it like this: No ☐ Yes ☒

If you make a mistake, simply scribble it out and mark the correct answer with a cross like this:

If you have any questions about the study please contact the SchoolNuts team on 03 8341 6266 or school.nuts@mcri.edu.au

I have read and understand the information letter and agree to take part in this project ☐

Your name: _____

Your child's name: _____

Name of your child's school: _____

Mobile telephone number: _____

This cover sheet will be kept separate to your questionnaire. We will remove your name and your child's name from any information you give us and we will use an identification number instead.

ID #: 077.2798.01

*** PLEASE COMPLETE THIS QUESTIONNAIRE AND THE PARENT CONSENT FORM AND GIVE THEM BOTH TO YOUR SON/ DAUGHTER TO RETURN TO SCHOOL AS SOON AS POSSIBLE ***

ID #: 077.2798.01

In this questionnaire we ask questions about your child, your family and their health.

Please answer ***all*** questions

DEMOGRAPHICS

1. What is your child's date of birth?

   _____/_____/_____ (DD/MM/YYYY)

2. What is your child's gender?

   Male ☐ Female ☐

3. What is your relationship to your child?

   Mother ☐ Father ☐ Other ☐

   (If other, please specify _____)

4. In what country was your child born?

   Australia ☐
   Other ☐
   (please specify _____)
   If other, how many years ago did the child move to Australia? _____(years)

5. In what city/town was your child born?

   _____

6. In what country was your child's **MOTHER** born?

   Australia ☐
   Other ☐
   (please specify _____)

7. In what country was your child's **FATHER** born?

   Australia ☐
   Other ☐
   (please specify _____)

8. What is the ancestry/ethnic origin of your child's **MOTHER**?

   British/Irish ☐
   European ☐
   Asian ☐
   Aboriginal or Torres Strait Islander ☐
   Don't know ☐
   Other ☐
   (please specify _____)

9. What is the ancestry/ethnic origin of your child's **FATHER**?

   British/Irish ☐
   European ☐
   Asian ☐
   Aboriginal or Torres Strait Islander ☐
   Don't know ☐
   Other ☐
   (please specify _____)

FOOD ALLERGY AND YOUR CHILD

10. Has your child **EVER** reacted to any of the following foods? *Not including foods that are labelled "may contain traces"*	**No**	**Yes**	**Never Eaten**
a. Peanuts	☐	☐	☐
b. Peanut butter	☐	☐	☐
c. Other nuts (eg: almond, hazelnut, cashew, brazilnut, pecan, pistachio, walnut)	☐	☐	☐
d. Hen's eggs (e.g. scrambled, fried, poached)	☐	☐	☐
e. Foods containing hen's eggs (e.g. quiche, biscuits, cakes, pancakes, egg custard)	☐	☐	☐
f. Cow's milk	☐	☐	☐
g. Dairy products made from cow's milk (e.g. cheese, yogurt, ice-cream)	☐	☐	☐
h. Sesame seeds on bread	☐	☐	☐
i. Sesame product (e.g. tahini, sesame paste, hommus, halva – excluding sesame oil)	☐	☐	☐
j. Fish	☐	☐	☐
k. Fish products (e.g. fish paste)	☐	☐	☐
l. Shellfish (e.g. prawns, lobster, crab)	☐	☐	☐
m. Soy or soy products (e.g. tofu, soy milk, soy sauce)	☐	☐	☐
n. Other 1 _____	☐	☐	☐
o. Other 2 _____	☐	☐	☐

A food allergy is a reaction to food that involves the immune system. Symptoms usually occur very quickly, minutes after eating a food and can include itching, swelling of the lips/tongue, breathing difficulties and vomiting and/or diarrhoea

	No	**Yes**	**Don't know**
11. Has your child **EVER** had a food allergy?	☐	☐	☐
12. Has your child **EVER** had an episode of food allergy-related anaphylaxis? (difficulty or rapid/noisy breathing, coughing, hoarse voice or collapse)	☐	☐	☐
13. Does your child **CURRENTLY** have a food allergy?	☐	☐	☐
14. Does your child **CURRENTLY** have an EpiPen or an Anapen?	☐	☐	☐
15. Does your child **CURRENTLY** have a written emergency action plan for allergic reactions or anaphylaxis?	☐	☐	☐

		No	Yes	Don't Know
16.	Does your child **CURRENTLY** have a written risk management plan for allergy at school?	☐	☐	☐

Has your child ever any had testing for food allergy?

17.	A skin prick test for food allergy?	☐	☐	☐
18.	A blood test for food allergy?	☐	☐	☐
19.	Any other tests for food allergy? (e.g. Vega testing, Kinesiology, Radionics, Iridology, Hair analysis, Homoeopathy, Acupuncture, Reflexology, Allergy elimination techniques, etc.) *Please list* _____	☐	☐	☐

OTHER ALLERGIC CONDITIONS

		No	Yes	Don't know
20.	Has your child **EVER** had wheezing or whistling in the chest at any time in the past? *If yes*... What age did symptoms first start? _____(Years) Have they stopped? Y / N *(please circle one)* *If yes...* At what age did symptoms stop? _____(Years)	☐	☐ If "no" skip to Q.25	☐
21.	Has your child had wheezing or whistling in the chest in the past **12 MONTHS**?	☐	☐ If "no" skip to Q.25	☐

22.	How many attacks of wheezing has your child had in the past **12 MONTHS?**	☐ None	☐ 1-3	☐ 4-8	☐ 8-12	☐ >12

23.	In the past **12 MONTHS**, how often, on average, has your child's sleep been disturbed due to wheezing?	☐ Never woken with wheezing	☐ Less than one night per week	☐ One or more nights per week

		No	Yes	Don't know
24.	In the past **12 MONTHS**, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths?	☐	☐	☐
25.	Has your child **EVER** had asthma? *If yes*... What age did symptoms first start? _____(Years)	☐	☐	☐
	26.Does your child **STILL** have asthma? *If no...* At what age did symptoms stop? _____(Years)	☐	☐	☐

27.	Were you told by a health professional that your child had asthma? *If other, please specify* _____	☐ No	☐ Doctor	☐ Nurse	☐ Pharmacist	☐ Other

		No	Yes	Don't Know
28.	In the past **12 MONTHS**, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection?	☐	☐	☐
29.	In the past **12 MONTHS**, has your child's chest sounded wheezy during or after exercise?	☐	☐	☐
30.	In the past **12 MONTHS**, has your child taken any medication (medicines/pills/puffers) for wheezing or asthma?	☐	☐	☐
31.	Do you have a written plan which tells you how to look after your child's asthma?	☐	☐	☐
32.	In the past **12 MONTHS**, how many visits has your child made to the doctor (family doctor, general practitioner or specialist) for his/her wheezing or asthma?			

	a). For a wheezy episode?	☐ None	☐ 1-3	☐ 4-12	☐ more than 12
	b). For a regular "check-up" for asthma?	☐ None	☐ 1-3	☐ 4-12	☐ more than 12
33.	In the past **12 MONTHS**, how many visits has your child made to a hospital Casualty or Emergency Department because of his/her asthma?	☐ None	☐ 1	☐ 2	☐ More than 2
34.	In the past **12 MONTHS**, how many times has your child been admitted to hospital because of his/her wheezing or asthma?	☐ None	☐ 1	☐ 2	☐ More than 2
35.	In the past **12 MONTHS**, how many days (or part days) of school has your child missed because of wheezing or asthma?	☐ None	☐ 1-5	☐ 6-10	☐ More than 10

		No	Yes	Don't know
36.	Has your child **EVER** had an itchy rash that was coming and going for at least six months?	☐	☐	☐
37.	Has your child **EVER** had eczema? *If yes...* What age did symptoms first start? _____(Years) Have they stopped? Y / N *(please circle one)* *If yes...* At what age did symptoms stop? _____(Years)	☐	☐	☐
38.	Has your child **EVER** had a problem with sneezing or a runny or blocked nose when he/she DID NOT have a cold or the flu?	☐	☐	☐
39.	Has your child **EVER** had hay fever?	☐	☐	☐

YOUR CHILD'S GENERAL HEALTH

40. In the past **12 MONTHS**, how many times has your child visited….

	number of visits			
	0 times	**1-2 times**	**3-4 times**	**5+ times**
a. GP	☐	☐	☐	☐
b. Allergist	☐	☐	☐	☐
c. Other specialist doctor (please specify)_____	☐	☐	☐	☐
d. Hospital emergency department	☐	☐	☐	☐
e. Complementary health practitioner (please specify)_____	☐	☐	☐	☐

YOUR FAMILY'S HEALTH

41. Does your child have any brothers or sisters? No ☐ Yes ☐

If yes…. 42. What is their date of birth?

	(day / month / year)	Please tick if they are a half sibling
Brother 1	_____/_____/_____	☐
Brother 2	_____/_____/_____	☐
Sister 1	_____/_____/_____	☐
Sister 2	_____/_____/_____	☐
Other sibling 1	_____/_____/_____	☐
Other sibling 2	_____/_____/_____	☐

43. Has anyone in your child's immediate family ever had a food allergy?
No ☐ Yes ☐ Not sure ☐

If yes, who…		Please list the food(s)
Mother	☐	_____
Father	☐	_____
Brother 1	☐	_____
Brother 2	☐	_____
Sister 1	☐	_____
Sister 2	☐	_____
Other sibling 1	☐	_____
Other sibling 2	☐	_____

44. Has anyone in your child's immediate family ever had.... *(please mark all that apply)*

	no-one	child	mother	father	brother 1	brother 2	sister 1	sister 2	other sibling	other sibling
a. Asthma	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
b. Eczema	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
c. Hay fever/allergic rhinitis	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
d. Insect allergy (eg. bee sting, ant)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
e. Drug allergy	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
f. Latex allergy	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

LIFESTYLE FACTORS

45. How many people in your household regularly smoke (most days of the week)? _____

If yes...

46. How many cigarettes are smoked by household residents **outside** your home? _____ per week
(eg: in the garden)

47. How many cigarettes are smoked by household residents **inside** your home? _____ per week

48. Does anyone smoke in the same room as the child?
- ☐ Never
- ☐ Sometimes
- ☐ Usually

49. Which of the following pets do you **CURRENTLY** keep at your child's home?

	No	Kept outside only	Kept inside only	Kept inside and outside
a. cat	☐	☐	☐	☐
b. dog	☐	☐	☐	☐
c. bird	☐	☐	☐	☐
d. other (specify): _____	☐	☐	☐	☐

50. Which of the following pets did you keep at your child's home **during the first 5 years of your child's life**?

	No	Kept outside only	Kept inside only	Kept inside and outside
a. cat	☐	☐	☐	☐
b. dog	☐	☐	☐	☐
c. bird	☐	☐	☐	☐
d. other (specify): _____	☐	☐	☐	☐

If you don't have a pet…

51. Does your household avoid pets (or have pets been removed from the household) because of allergy?

No	☐
Yes, avoid cats	☐
Yes, avoid dogs	☐
Yes, avoid both cats and dogs	☐

52. What is the highest level of education achieved by your child's

	a. **mother**	b. **father**
Primary school	☐	☐
Secondary school	☐	☐
Certificate	☐	☐
Diploma/Advanced Diploma	☐	☐
Bachelor Degree	☐	☐
Graduate Diploma/Graduate Certificate	☐	☐
Postgraduate Degree	☐	☐
Other education_____	☐	☐
Don't know	☐	☐

53. Is your child's mother currently working? No ☐ Part-time ☐ Full-time ☐

If applicable, please indicate the type of work

Manager or administrator (eg. Magistrate, general manager, school Principal, director of Nursing)	☐
Professional (eg. Scientist, nurse, allied health professional, teacher, artist)	☐
Associate professional (eg: Technician, manager, police officer, small business owner)	☐
Tradesperson or related worker (eg. Hairdresser, gardener, florist)	☐
Advanced clerical service worker (eg. Secretary, flight attendant, law clerk, personal assistant)	☐
Intermediate clerical, sales or service worker (eg. Administration worker, child care or hospitality worker)	☐
Intermediate production or transport worker (eg. Machine operator, bus driver, sewing machinist)	☐
Elementary clerical, sales or service worker (eg. Filing/mail clerk, parking inspector, sales assistant)	☐
Labourer or related worker (eg. Cleaner, factory worker, farm hand, kitchen hand)	☐

54. Is your child's father currently working? No ☐ Part-time ☐ Full-time ☐

If applicable, please indicate the type of work

Manager or administrator (eg. Magistrate, general manager, school Principal, director of Nursing)	☐
Professional (eg. Scientist, nurse, allied health professional, teacher, artist)	☐
Associate professional (eg: Technician, manager, police officer, small business owner)	☐
Tradesperson or related worker (eg. Hairdresser, gardener, florist)	☐
Advanced clerical service worker (eg. Secretary, flight attendant, law clerk, personal assistant)	☐
Intermediate clerical, sales or service worker (eg. Administration worker, child care or hospitality worker)	☐
Intermediate production or transport worker (eg. Machine operator, bus driver, sewing machinist)	☐
Elementary clerical, sales or service worker (eg. Filing/mail clerk, parking inspector, sales assistant)	☐
Labourer or related worker (eg. Cleaner, factory worker, farm hand, kitchen hand)	☐

YOU HAVE REACHED THE END OF THE QUESTIONNAIRE!

THANK YOU FOR PARTICIPATING ☺

Please give this questionnaire and the parent consent form to your child to return to school.