Environmental and genetic risk factors for food allergy in children with Asian ancestry

Noor Hidayatul Aini Bte Suaini

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Department of Paediatrics
Faculty of Medicine, Dentistry and Health Sciences
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Abstract

Epidemiological and genetic studies on food allergy to date have focused primarily on the Caucasian population. This is despite emerging evidence that food allergy appears to be rising in Asian countries, alongside the increasing Westernisation and urbanisation in these countries. Even less is known about Asian migrants living in Western countries. A population-based study on food allergy found the risk of food allergy to be three times higher in infants of East Asian ancestry than infants of Caucasian ancestry. It is thought that infants growing up in Australia are exposed to environmental agents that have a differential effect on the immune system depending on their genetic background. However, the influence of specific genetic and environmental risk factors is currently unknown. It is also unclear if the increased risk of food allergy translates to higher risk of other allergic diseases later in childhood.

The main objective of this thesis is to identify specific environmental and genetic factors on the risk of food allergy in the Asian population living in Australia. This thesis aims to quantify the prevalence of, and identify risk factors for food allergy and allergic comorbidities in the Asian population. An additional aim is to identify genetic variants that increase the risk of food allergy in the East Asian population and compare this to the Caucasian population living in Australia.

This thesis primarily used data and samples from the longitudinal HealthNuts study where 5,276 1-year-old infants attending council run vaccination sessions across Melbourne were recruited. Skin prick tests to a range of food were carried out on infants and those with a wheal size ≥ 1 mm underwent an oral food challenge. The 1-year-old infants were followed up again at age 6 years and data collected at this follow-up visit were also used for analyses in this thesis. Additionally, data collected from a Growing Up in Singapore Towards Healthy Outcomes (GUSTO) birth cohort was also used to compare the risk factors and prevalence of allergic diseases between East Asians living in Melbourne and East Asians living in Singapore.

This thesis reports that Australian-born children with East Asian parents have a higher burden of allergic rhinitis, eczema and aeroallergen sensitisation but not asthma, in the first six years of life compared to children of Caucasian ancestry. Moreover, children with IgE-mediated food allergy and eczema in infancy were 3 times more likely to have asthma and 2 times more likely to have allergic rhinitis at age 6 years, irrespective of ancestry. Additionally, East Asian children living in Melbourne have a higher risk of food allergy compared to East Asian children living in Singapore. Despite delayed introduction of allergens into the diet compared to the Asian population in Melbourne, Asian children in Singapore had less food allergy. While eczema rates were lower in Singapore than in Melbourne, early onset eczema was associated with an increased risk of food allergy in both Singapore and Melbourne.

In terms of genetic risk factors, a systematic review conducted as part of this thesis identified several genes of interest known to be involved in immune regulation, cell function and epidermal barrier function. However, studies were of varied quality and the reproducibility of findings for the same SNPs were minimal. Some of the highly reproducible genes identified from the literature include *HLA, FLG* and *IL13*. Additionally, there was also a paucity of studies carried out in the Asian population that were able to elucidate underlying mechanisms for the differential food allergy risks observed in the population. This highlighted the need for genetic studies focused in this population. This thesis found that *HLA* rs7192 minor allele was associated with increased risk of peanut allergy in the Caucasian population but not East Asian population. Among sensitised children with two East Asian born parents, those with the minor allele for rs231735, rs231804 or rs11571291 (all *CTLA4*) have a reduced risk of egg allergy.

The findings of this thesis identify Asian children living in Australia as a high risk allergic group not just in infancy but throughout early childhood. As a multifactorial disease, both environmental and genetic factors are known to contribute to the pathogenesis of food allergy. Therefore, it may be that the increased risk of food allergy observed in genetically predisposed East Asian children living in Melbourne unmasked upon exposure to environmental risk factors.

Declaration

This is to certify that:

i. the thesis comprises only my original work towards the PhD except where indicated in the Preface,

ii. due acknowledgement has been made in the text to all other material used,

iii. the thesis is fewer than 100 000 words in length, exclusive of tables, maps, bibliographies and appendices.

Signed: _____ Date: 15/06/2019

Noor Hidayatul Aini Bte Suaini

Preface

This thesis is made up of my own work under the supervision of my supervisory panel (Professor Katrina Allen, Associate Professor Justine Ellis, Dr. Jennifer Koplin and Dr. David Martino) except where indicated below:

HealthNuts study

The PhD project forming this thesis is part of a larger study, HealthNuts study. The HealthNuts study was developed and conducted by Professor Katrina Allen (Principal Investigator in collaboration with Professor Mimi Tang, Professor Shyamali Dharmage, Associate Professor Lyle Gurrin, Dr. Nicholas Osborne, Professor Melissa Wake, Professor Anne-Lousie Ponsonby, Dr. Melanie Matheson, Dr. Adrian Lowe, and Dr. David Hill.

Research nurses and research assistants from the Population Allergy group (Murdoch Children's Research Institute) carried out recruitment of participants and administration of questionnaires for the HealthNuts study.

Data cleaning for the baseline data (12-months data point) used in this thesis was done by Dr. Jennifer Koplin, Dr. Pamela Martin and Dr. Nicholas Osborne. I was involved in the data cleaning of the age 4 and age 6 years follow-up questionnaires along with Dr. Rachel Peters and Dr. Mari Sasaki. I was also responsible for the data cleaning of biological specimen records.

Work within this thesis

The study plans for the epidemiological sections of this thesis were planned together with Professor Katrina Allen and Dr. Jennifer Koplin.

Chapter 4 of this thesis is a collaborative study carried out between the Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort in Singapore and our HealthNuts study. Initial collaboration was initiated by Dr. Jennifer Koplin. Thereafter, I carried out subsequent collaboration and data transfer agreements. Preliminary analyses were carried out in each study independently and I was responsible for the data analyses within HealthNuts. I was also responsible for carrying out the follow-up analyses on the combined dataset which involved sharing and merging of raw data from the two cohorts.

Statistical plans were formulated together with the primary investigators of each study - Dr. Elizabeth Tham, Dr. Evelyn Loo, Dr. Lee Bee Wah and Professor Lynette Shek from the GUSTO team and Professor Katrina Allen, Dr. Jennifer Koplin and Dr. Rachel Peters from the HealthNuts study.

In terms of the genetic aspect of the study, study plans were formulated in conjunction with Associate Professor Justine Ellis and Dr. David Martino.

I was involved in the processing and storage of biological samples obtained from the HealthNuts participants during the period of April 2015 to July 2016. Existing DNA samples required for the project were previously extracted by Dr. Tina Tan (n=281). I extracted DNA from the remaining 206 biological samples required for this thesis and quantified the concentration of all DNA samples. DNA extractions from newborn screening cards were carried out using the Zymo DNA Extraction kit. The protocol used for the Zymo extraction was adopted from Dr. Jane Loke who optimised and modified the manufacturer's protocol for use on newborn screening cards. Mr Darren Suryawijaya and Ms. Yichao Wang assisted with preparation of a subset of samples for DNA extractions.

I designed the genotyping assay for the list of genes to be genotyped using the online Agena Bioscience™ Assay Design platform with assistance from Associate Professor Justine Ellis and Dr. David Martino. I also carried out all genotyping and data analyses.

Dr. Benjamin Ong (MCRI) and Ms. Alisha Monaco (VCGS) assisted with the handling of the Agena Bioscience™ MassARRAY System facility to obtain genotyping data.

This thesis includes two publications of which I am a primary author and contributed more than 50% of the work. The contributions of all persons involved in these multi-authored publications are as follows:

For the first publication included as Chapter 3, the conception and design of the study were planned together with Dr. Jennifer Koplin, Dr. Rachel Peters and Professor Katie Allen. All named co-authors either provided statistical support for analyses of data and/or contributed to the interpretation of results. All co-authors also reviewed the manuscript draft critically for intellectual content and approved the final version to be published.

For the second publication included as Chapter 5, I developed the protocol, search strategy and risk of bias assessment under the guidance of Dr. Jennifer Koplin and Associate Professor Justine Ellis. Additionally, I reviewed all titles and abstracts for eligibility against a pre-determined set of inclusion criteria as well as extracted data from the full text of the original papers, including carrying out the quality assessment of included studies. Co-authors, Ms. Yichao Wang and Ms. Victoria Soriano, checked the accuracy and authenticity of data extracted. Dr. David Martino and Professor Katie Allen contributed to the data analysis and interpretation of data. All authors contributed to the drafting and revising of the article for intellectual content and approve the final version of the manuscript.

Publication status

Thesis chapter	Publication status	Publication
3	Published by *Journal of Allergy and Clinical Immunology* on 1st February 2019.	Children with East Asian-born parents have an increased risk of allergy but may not have more asthma in early childhood.
4	Unpublished material not submitted for publication. Preparation for a written manuscript in progress.	Not applicable
5	Accepted by *Allergy* on 18th February 2019.	Genetic determinants of paediatric food allergy: A systematic review
6	Unpublished material not submitted for publication.	Not applicable
7	Unpublished material not submitted for publication.	Not applicable

Other manuscripts published during candidature

1. Wang Y, Allen K, Suaini NH, McWilliam V, Peters R, Koplin J. 2018, The global incidence and prevalence of anaphylaxis in children: A systematic review. Allergy. 2019 [Epub ahead of print]
2. Wang Y, Allen KJ, Suaini NHA, Peters RL, Ponsonby AL, Koplin JJ. Asian children living in Australia have a different profile of allergy and anaphylaxis than Australian-born children: A state-wide survey. Clin Exp Allergy. 2018; 48 (10):1317-24.
3. Koplin JJ, Suaini N, Vuillermin P, Ellis J, Panjari M, Ponsonby A-L et al for the HealthNuts Study Investigators. 2015, Polymorphisms affecting vitamin D binding protein modify the relationship between serum vitamin D and food allergy. Journal of Allergy and Clinical Immunology. Aug 7, doi: 10.1016/j.jaci.2015.05.051.
4. Suaini N, Zhang Y, Vuillermin P, Allen K, Harrison L. 2015, Immune modulation of vitamin D and its relevance to food allergy. Nutrients. Jul 27, doi: 10.3390/nu7085271

Conference Presentations

I have presented in conferences both locally and internationally during my candidature. All of my abstracts submitted to conferences were chosen as oral presentations.

1. Suaini N, Tham EH, Koplin JJ, Loo EXL, Peters RL, van Bever H, Martino DJ, Goh AEN, Ellis JA et al. Asian children living in Melbourne have a higher prevalence of food allergy than those living in Singapore despite delayed introduction of allergens in the latter. This abstract was presented at the Food Allergy and Anaphylaxis Meeting (FAAM) 2018

2. Suaini N, Allen KJ, Wang Y, Soriano V, Martino DJ, Ellis JA, Koplin JJ. Genes associated with food allergy: A systematic review. This abstract was also presented at the Food Allergy and Anaphylaxis Meeting (FAAM) 2018

3. Suaini N, Koplin JJ, Ellis J, Martino DJ, Dharmage SC, Lowe AJ, Tang MLK, Ponsonby AL, Gurrin LC, Wake M, Allen KJ, for the HealthNuts study group. Children with Asian-born parents living in Melbourne have more allergy in the first six years of life. This abstract was presented at the 5th Pediatric Allergy and Asthma Meeting (PAAM) in 2017.
    a. One out of five winners for the Abstract Award

4. Suaini N, Koplin JJ, Ellis J, Matheson M, Allen K. Identification of candidate genes associated with the development of food allergy. It was presented at:
    a. Murdoch Children's Research Institute Research Student Association (RSA) Student Symposium 2016
    b. Australian Society for Medical Research 2016 Victorian Student Research Symposium

Scholarships and funding

The following awards funded me during my PhD candidature:

1. Centre for Food and Allergy Research (CFAR) postgraduate scholarship to undertake my PhD (April 2015 to Dec 2018).
2. Travel award to attend the Global Young Scientists Summit in Singapore, awarded by Contact Singapore/National Research Foundation (January 2017).
3. Henry and Rachel Ackman Travelling Scholarship awarded by the Department of Paediatrics, University of Melbourne (2017) to attend 5th Pediatric Allergy and Asthma Meeting (PAAM2017)
4. Travel grant to attend PAAM2017, awarded by the European Academy of Allergy and Clinical Immunology (EAACI)
5. Continuing Education Scholarship Travel Grant awarded by Centre for Food and Allergy Research (CFAR), 2018 to attend Food Allergy and Anaphylaxis Meeting (FAAM2018)
6. Travel grant to attend FAAM2018, awarded by the European Academy of Allergy and Clinical Immunology (EAACI)

7. Student Conference Support Scholarship awarded by Murdoch Children's Research Institute (MCRI), 2018 to attend FAAM2018

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This PhD would not have materialised without the support and encouragement of a big support network group whom I would like to acknowledge.

I would firstly like to express my deepest gratitude to my supervisory panel Professor Katie Allen, Associate Professor Justine Ellis, Dr. Jennifer Koplin and Dr. David Matino, not only for their guidance and advice on the technicalities of the project but also for their emotional support throughout my PhD candidature. I am ever so fortunate to have a great supervisory team that supports me in my endeavours. Thank you, Katie, for always being so understanding whenever I needed to attend to family matters overseas and for nurturing me to grow as a research scientist. I would never had started on this journey if you had not accepted me as your honours student many years ago.

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Jen and Dave, I am always thankful to the both of you for always having your door wide open for me to drop in whenever I needed clarifications. For helping me place the PhD problems I faced into perspective and reassuring me that all PhD students go through the same ups and downs throughout their candidature, it makes me feel better knowing that I was not alone. Thanks Dave for still being ever so present and available to guide me even after you have left MCRI. And to Jen, no words could ever describe the significance your presence has been in my PhD journey. For being so easy going yet nurturing and guiding at the same time, I always rave about how great you are as a supervisor.

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Loke and Dr. Rachel-Chiaroni Clarke for taking the time to be a part of my advisory panel and providing suggestions and recommendations for my project.

I would also like to express a special appreciation to the HealthNuts team. The HealthNuts study would not have been here without the hard work and dedication of the team of research assistants and nurses. Having been part of the group since 2012, the team feels like home now. I would like to particularly mention Giovanni, Helen, Mari, Mark, Melanie, Michael, Rachel, Vicki and Yichao for your help with reviewing some of my materials, giving advice on navigating the admin matters of submission or simply listening to me rant. The PhD journey was so much more bearable with all of you around. A special mention also to Sarah, I am so appreciative of you for always promptly replying and patiently helping me with my never-ending questions even when you are miles away.

Last but not least, on a personal note, I wish to thank my family, relatives and friends who have been by my side throughout this long, arduous journey. Mum and Dad, thank you for all your prayers and to my siblings as well for always being so willing and ready to fly over to be by my side whenever I was going through tough moments.

To my close friends here at MCRI and Melbourne - Rachel, Ryan, Posh, Isaac, David, as well as members of the weekly Saturday writing group and other PhD peers who have also been through this journey with me and sharing my pain, encouraging me along the way. I am so glad I had all of you to cry and share my joys with. Jane, for being someone I could turn to both professionally and personally, you are a special one and I will always be indebted to you. Finally, my distant friends in Singapore who stayed by my side despite me having been out of Singapore for 10 years now, for being so supportive and giving me your encouragements virtually from miles away, it has been immensely helpful having all of you still to turn to.

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Figure 1.1 Global regional variation of food allergy	Renz H, Allen KJ, Sicherer SH, et al. Food allergy. *Nat Rev Dis Primers.* 2018;4:17098	Yes
Figure 1.2 Summary of food allergy mechanism	Renz H, Allen KJ, Sicherer SH, et al. Food allergy. *Nat Rev Dis Primers.* 2018;4:17098	Yes
Figure 1.3 The role of hygiene hypothesis, dual allergen hypothesis and vitamin D hypothesis in induction of oral tolerance.	du Toit G, Tsakok T, Lack S, Lack G. Prevention of food allergy. *J Allergy Clin Immunol.* 2016;137(4):998-1010.	Yes
Figure 2.2 Summary of recruitment and assessments in each HealthNuts follow-up study	Koplin JJ, Wake M, Dharmage SC, Matheson M, Tang ML, Gurrin LC, et al. Cohort Profile: The HealthNuts Study: Population prevalence and environmental/ genetic predictors of food allergy. Int J Epidemiol. 2015;44(4):1161-71.	Yes
Figure 2.4 Summary of the iPLEX gold reaction processes	Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. Curr Protoc Hum Genet. 2009;60(1):2.12.1-2.12.8.	Yes

Abbreviations

AAP	American Academy of Paediatrics
AIM	Ancestry Informative Markers
ASCCEG	Australian Standard Classification of Cultural and Ethnic Groups
ASCIA	Australasian Society for Clinical Immunology and Allergy
CI	Confidence Interval
CNV	Copy Number Variations
CNVR	Copy Number Variations Region
DC	Dendritic Cells
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
EAT	Enquiring About Tolerance
FCS	Fetal Calf Serum
FEV	Forced Expired Volume
FLG	Filaggrin
FVC	Forced Vital Capacity
g	Centrifugal force
GOFA	Genetics of Food Allergy Study
GUSTO	Growing Up in Singapore Towards healthy Outcomes
GWAS	Genome Wide Association Study

HI-FCS	Heat-Inactivated Foetal Calf Serum
HLA	Human Leukocyte Antigen
HREC	Human Research Ethics Committee
HWE	Hardy Weinberg Equilibrium
Ig	Immunoglobulin
IL-	Interleukin
ISAAC	International Study of Asthma and Allergies in Childhood
LEAP	Learning Early About Peanut allergy
MAF	Minor Allele Frequency
MALDI	Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight
MCRI	Murdoch Children's Research Institute
MDS	Multidimensional Scaling
mL	Millilitre
NBS	Newborn Screening Cards
ng	Nanograms
NHMRC	National Health and Medical Research Council
nmol	Nanomole
OFC	Oral Food Challenge
OR	Odds Ratios
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate Buffer Saline

PC	Principal Components
PCR	Polymerase Chain Reaction
PR	Prevalence Ratios
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	Prospective Register of Systematic Reviews
RCH	Royal Children's Hospital
SACC	Standard Australian Classification of Countries
SAP	Shrimp Alkaline Phosphatase
SEIFA	Socio-Economic Indexes for Areas
SNP	Single Nucleotide Polymorphism
SPT	Skin Prick Test
Th	T-helper cell
TLR	Toll-Like Receptor
TSLP	Thymic Stromal Lymphopoietin
UEP	Unextended Extend Primers
UTR	Untranslated Region
UVR	Ultraviolet Radiation
VCGS	Victorian Clinical Genetics Services
WHO	World Health Organisation

Chapter 1 Literature review

1.1 Introduction to food allergy

Food allergy is a health problem of international significance. It involves the breakdown of clinical and immunological tolerance against ingested foods, which manifests clinically as hives, wheezing and angioedema (1). It is an immune- mediated disease that can act through Immunoglobulin E (IgE) or non-IgE mechanisms. Little is known about non-IgE-mediated food allergy and it is poorly characterised clinically. Conversely, IgE-mediated food allergy is the most common and best characterised form of food allergy. It is the only type that can lead to a life threatening allergic reaction known as anaphylaxis. There is currently no cure for IgE-mediated food allergy apart from strict avoidance of the relevant allergenic food. This thesis is focused on IgE-mediated food allergy, herein addressed simply as 'food allergy'.

While the economic cost of allergies in Australia was estimated to be 7.8 billion in 2007 (2), the economic cost of food allergies, specifically, is not known. In the United States, food allergic reactions and anaphylaxis resulted in an estimated economic burden of $510 million in 2007 (3). More recent evidence has shown that it costs $24.8 billion annually or $4184 per child, with $4.3 billion alone dedicated to direct medical costs for food allergy (4). In Europe, the additional total household costs were higher by €3961 for households with a child diagnosed with food allergy and €4792 for households with adolescents diagnosed with food allergy (5). The high burden of the economic cost and implications on quality of life that allergies can have highlights a pressing need for research in the field to work towards better prevention and management of food allergy.

In Australia, it is anticipated that 7.7 million people will have an allergic disease by 2050, an increase of 70% from the reported 4.1 million people in 2007 (2). However, the global prevalence of food allergy differs geographically suggesting a strong role for the contribution of environmental factors. Over the last 30 years community rates of food allergy have risen dramatically in developed countries, with developing countries undergoing urbanisation, now showing concerning trends (6). Differential rates of food allergy have

also been observed in different populations, with variations particularly between the Caucasian and Asian population (7).

In order to reduce the burden of food allergy on the society and improve quality of life of children living with food allergy, it is vital to gain a better understanding of the risk factors for food allergy. This will also help devise potential measures to prevent the development of food allergy.

1.2 Diagnosis and clinical features of food allergy

According to the European Academy of Allergy and Clinical Immunology (EAACI) guideline, the gold standard measure for the diagnosis of food allergy is through oral food challenges (8). Double-blinded placebo controlled oral food challenge (OFC) is the accepted gold standard, however, OFCs have been shown to be sufficient for diagnosing objective symptoms in children (9, 10). OFCs involve giving increasing doses of food to the patient until the onset of adverse clinical reactions. OFCs need to be done under medical supervision due to the risk of anaphylaxis, making it costly to be carried out since it requires specialised personnel. Given its costly and risky nature, skin prick tests (SPT) and/or specific IgE measurements to identify clinical reactivity to food allergens, along with detailed clinical history, are often used instead to confirm food allergy status.

However, SPT or specific IgE results alone are not a good indicator of food allergy status and are at best only a measure of food sensitisation. A sensitised individual may be able to consume a particular food without a reaction (tolerant). Food allergy is only confirmed when a sensitised individual experiences symptoms upon consumption of food in question. For example, in one study only 22% (19/85) of peanut sensitised children had true peanut allergy (11). Accurately diagnosing food allergy status is therefore crucial.

As an immune-mediated disease, clinical reactions associated with food allergy can involve the respiratory, gastrointestinal, cardiovascular systems and the skin. Symptoms typically occur within minutes of ingestion although sometimes delayed reactions up to several hours can occur. Such symptoms include hives, angioedema of the lips, tongue swelling, wheezing, coughing, and tachycardia. In its most severe form, anaphylaxis involves several organ systems and induce life-threatening hypovolemic shock or respiratory compromise (1).

Given the severity of adverse reactions, the first occurrence of adverse reactions to food can be daunting and overwhelming for families. Managing food allergy can be taxing on families and can have a detrimental effect on quality of life as strict avoidance of the food is the only way to treat food allergy (12). This makes patients susceptible to accidental exposure. Those with severe food allergy and at risk of anaphylaxis are prescribed epinephrine auto injectors, where in the case of accidental exposure, epinephrine is then administered by intramuscular injection into the lateral thigh.

1.3 Prevalence of food allergy

Prescription rates of epinephrine auto injectors (13) and rates of anaphylaxis admissions to emergency departments (14) are commonly used as a measure to estimate the prevalence of food allergy. In this regard, the prevalence of food allergy is thought to be rising over the past decade, marked by an increase in hospital admissions due to food-induced anaphylaxis (15). However, such proxy measures of food allergy do not provide an accurate estimate on the incidence of food allergy. Rather, they reflect an increase in severity of reactions and not increasing prevalence. The perceived increase in prevalence reflected by these proxy measures may also be due to better screening and recognition of food allergy symptoms. Although there is a lack of comprehensive studies to support an increase in prevalence, there is still a widespread consensus among health professionals and the research community that the prevalence of food allergy has been increasing over the last twenty years (1, 7).

Estimating the global prevalence of food allergy is challenging and current estimates vary (Figure 1.1). An illustration of the global prevalence and patterns of food allergy were captured in a collaborative project carried out by the World Allergy Organisation (WAO) (7). Out of 89 countries included in the survey, only nine had accurate prevalence data based on OFC. The highest prevalence based on OFC was reported in Australia, with 10% of 1-year old infants having food allergy (7, 16-18). Challenge-proven food allergy prevalence in Asian countries such as Thailand was lower at 1% (7). Based on SPT and history of reaction, Singapore reported an equivalently low prevalence of food allergy at 2.9 % in 12-month old infants (19). China, surprisingly had comparable rates (ranging from

3.8% to 7.7%) to European countries (7). The geographical variation in food allergy prevalence strongly suggests a role for local environmental factors in the development of food allergy.

Apart from variations in prevalence globally, variations in prevalence between different populations within the same geographical location have also been observed. Of note, a state-wide School Entrant Health Questionnaire carried out in primary schools in Victoria, Australia, found that among children with Asian ethnicity, those born in Asia had a lower risk of nut allergy than those born in Australia (20). However, among Australian-born children, children with Asian ethnicity were more likely to have nut allergy than children with Caucasian ethnicity. A study in the United States (US) reproduced similar findings (21). Children born outside of US had a lower risk of food sensitisation than those born in US. When compared among those born in US, children of migrant parents had a higher risk of food sensitisation. In this study, classification of migrant parents was based on the nativity of the head of household which was defined as either US-born or foreign-born. Another study in the United Kingdom (UK), found an over-representation of non-Caucasian population (56%) in the paediatric allergy clinic, of which 32.9% were Asian/Asian British (22). The non-Caucasian group was also found to have a significantly higher number of allergen sensitisations per child, with a mean of 2.05 sensitised allergen per non-Caucasian child compared to 1.22 sensitised allergen per Caucasian child. Together, these studies support the finding that food allergy prevalence differ between children of migrant parents and children of locally born parents. However, no direct comparisons of risk factors for food allergy between the same ethnic population living in different geographical locations have been performed before (e.g Asians living in Asian countries versus Asians living in Western countries).

There exist great variations in estimates across the world due to the various ways of measuring prevalence, making prevalence estimates of food allergy challenging. While we know from current literature that there are indeed differences in food allergy prevalence, in particular between the Asian and Caucasian population, it is unclear if these differences persist into later childhood and into adolescence. Research into this area could potentially help identify individuals and populations most at risk of food allergy for implementation of targeted treatment and prevention strategies.

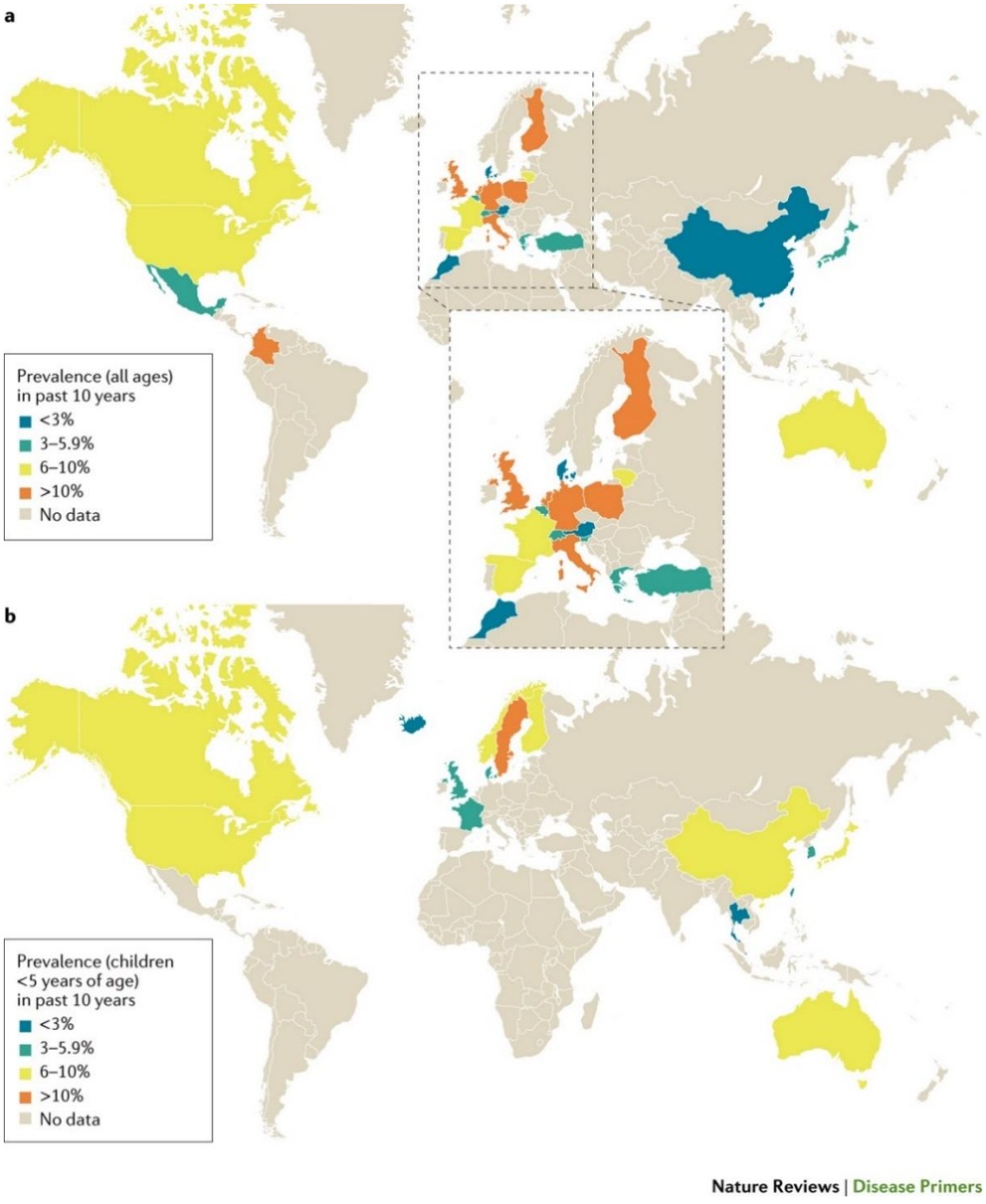

Figure 1.1 Global trends of food allergy prevalence. a.) Prevalence of food allergy in all ages. b.) Prevalence of food allergy in children under 5 years. Current evidence shows a global regional variation of food allergy. Figure sourced from Renz et al (1).

1.4 Natural history of food allergy

The resolution of food allergy is known to differ according to the allergen. Most children do outgrow their food allergies, particularly egg and cow's milk allergy while peanut and tree nuts tend to persist for life (23). Challenge-proven egg allergy was shown to resolve in 47% of children from the HealthNuts study, a longitudinal, population-based study of food allergy in 12-month-old infants in Melbourne, Australia (24). Similar estimates of egg allergy resolution were reported by the EuroPrevall birth cohort, comprising of 12,049 infants (0 - 2 years old) from nine European countries (25). With an incidence of 1.23%, 50% resolved their egg allergy within a year after diagnosis. In a retrospective study of Japanese children with egg allergy, 30% (66/226) achieved tolerance by 3 years of age and 73% (164/226) achieved tolerance at 6 years of age (26). In comparison, peanut allergy resolved by age 4 years in 22% of children with challenge-confirmed peanut allergy at age 1 year (27). Peanut allergy was also found to persist until young adult life as shown by the Isle of Wight birth cohort which followed up infants to 18 years (28). Given the persistence of peanut allergy into later in life, it is no surprise that food allergy research tended to focus on resolving peanut allergy over other subtypes of allergy.

1.5 Mechanisms of food allergy

To improve knowledge on the aetiology of food allergy, it is crucial to understand the mechanisms underlying its development. This section will briefly explore the mechanisms of action and immunological tolerance to food allergy.

Upon ingestion of food, food proteins are broken down by digestive enzymes. Any remaining food proteins are transported to the mucosa through gut epithelial cells or M cells located above Peyer's patches, which are then taken up by dendritic cells (DCs) (29). The uptake of food proteins by DCs would normally lead to oral tolerance. Tolerogenic DCs process the proteins and present them to allergen-specific naïve CD4+ T cells in the gut-draining lymph nodes. The type of DCs activated and co-stimulatory molecules expressed determine the consequent immune responses. For oral tolerance, these naïve CD4+ T cells differentiate into T helper 1 (Th1) cells or regulatory T cells (Tregs) (30, 31) which produce antigen-specific IgG and does not cause an allergic response. Failure to mediate oral tolerance, would lead to allergic sensitisation.

Sensitisation refers to a state where an individual produces detectable allergen-specific IgE, a hallmark of atopic predisposition. In sensitised individuals, the naïve CD4+ T cells would instead differentiate into T helper 2 (Th2) cells that would result in the production of cytokines such as interleukin 4 (IL4), interleukin 5 (IL5) and interleukin 13 (IL13). The production of these cytokines would initiate allergen-specific IgE production, thereby inducing IgE class switching in B cells and plasma cells (32). It is important to note that sensitisation alone is not equivalent to clinical food allergy. It is possible to be sensitised to a particular food without having any clinical symptoms of food allergy. In order to confirm food allergy, an OFC will need to be carried out (described in section 1.1.1).

Once sensitised, subsequent exposure to the relevant antigen can lead to cross-linking of IgE molecules on the surface of mast cells. This cross-linking induces the release of pro-inflammatory mediators through basophil degranulation, leading to the clinical signs and symptoms that we observed in food allergy (1) (Figure 1.2). These clinical symptoms can occur within minutes and usually within one hour of exposure to the food (1). Recent developments in understanding the underlying cellular mechanisms of food allergy have led to studies investigating specific treatments to induce loss of sensitisation or increase tolerance.

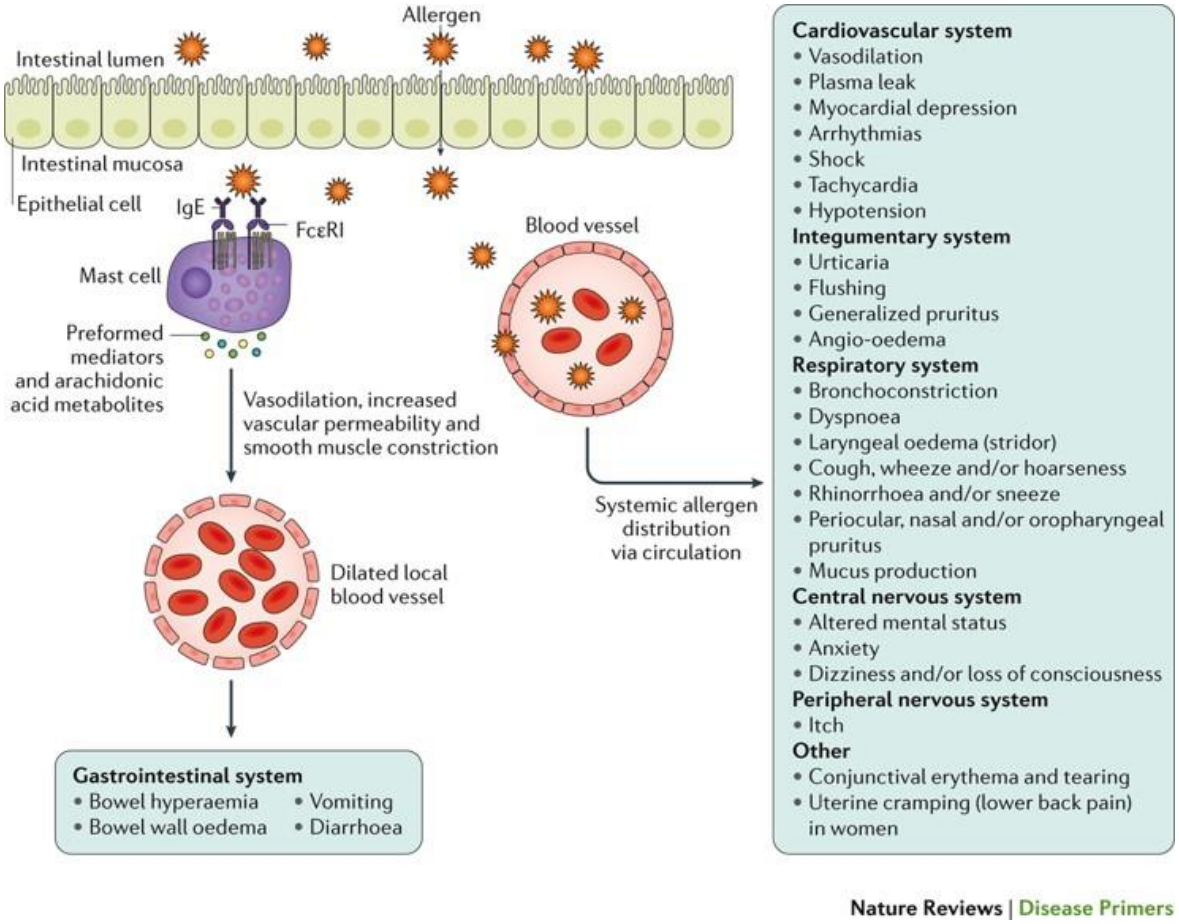

Figure 1.2 Summary of food allergy mechanism. Food allergens ingested passed through the intestinal lumen and encounter mast cells in the mucosa. On recognition by immunoglobulin E (IgE) antibodies, these allergens bind to IgE antibodies bound to mast cells, resulting in cross-linking. This cross-linking causes the release of preformed mediators and arachidonic acid metabolites, which promote a series of physiological changes leading to the clinical symptoms and signs observed in those with food allergy. Sourced from Renz et al (1).

1.6 Co-existence with other allergic diseases

Food allergy is also often developed alongside other allergic diseases such as eczema, asthma and allergic rhinitis. Food allergy has been shown to be a risk factor for allergic rhinitis and asthma, independently of eczema. Food allergy was associated with the development of asthma (OR 2.16; 95% CI: 1.94-2.40), and rhinitis (OR 2.72; 95% CI, 2.45-3.03) in a retrospective birth cohort (33). This association remained significant in children without eczema. Moreover, a meta-analyses of birth cohorts revealed that early life food sensitisation is associated with an increased risk of wheeze/asthma (pooled OR 2.9; 95% CI 2.0-4.0), eczema (pooled OR 2.7; 95% CI 1.7-4.4) and allergic rhinitis (pooled OR 3.1; 95% CI 1.9-4.9) from 4 to 8 years (34). This association was further confirmed by two independent birth cohorts which found an association between food sensitisation at 2 years and subsequent asthma and allergic rhinitis by age 10-12 years. (35). However, a major challenge for such studies is the potential confounding brought about by a history of eczema and family history of atopy.

On the other hand, another school of thought alludes that eczema in infancy precedes food allergy and the development of asthma and allergic rhinitis later in childhood (36). Mechanistically, defects in epithelial barrier protection is thought to promote allergen entry through the skin and contribute to eczema followed by asthma (37, 38). This natural progression of atopic manifestations is often quoted as the ‘allergic march’ or ‘atopic march’. It has been contended that atopic march is often viewed at the population level but rarely seen at the individual level (39). Current literature has also shown that these allergic diseases share a genetic makeup, lending further support to the notion of atopic march (40-44).

Nonetheless, it is arguable that the atopic march may likely be an oversimplification of a seemingly complex and multifactorial process of allergic disease progression (45). Adding to the complexity is the unknown implication ethnicity may have on atopic march; whether the pattern of atopic march and prevalence of other allergic diseases such as asthma, eczema, allergic rhinitis and allergic sensitisation differs by ethnicity.

1.7 Food allergy is a complex disease

Food allergy has long been recognised as a multifaceted, complex disease where both environmental and genetic factors contribute to its pathogenesis. Current evidence showing differential risks of food allergy in Asian and Caucasian population raises two central questions. Why do Asians in Australia have more food allergy than Asians in Asia? And, why do Asians in Australia have more food allergy than Caucasians in Australia? These two questions further demonstrate that both genetics and environmental factors play a role in the risk of developing food allergy.

Presumably with similar genetics, the observation that Asians in Australia have more food allergy than those in Asia highlights the potential influence that differences in environmental factors can have on food allergy risk. It is likely that upon migration to Australia, the removal of protective risk factors in the environment present in the Asian country, increases the risk to food allergy in the new country. There is mounting evidence that exposures during early life can help foster the development of tolerance. This critical period in infancy is known as the 'window of opportunity'. The window of opportunity refers to the first 1000 days in early life that spans from intrauterine development through to the first 2 years of life (46). Environmental exposure during this critical period determines the induction of oral tolerance, which is perceived to influence the susceptibility of developing allergies and diseases later in life (1). Key environmental exposure that can mediate oral tolerance are discussed in the next section below.

The finding that Asians in Australia have more food allergy than Caucasians in Australia, where the environment in question is well-matched, suggest the potential influence of genetic background. A major challenge with studying the genetics of food allergy is the polygenic nature of the disease; with low penetrance and variable expressivity. This makes it incredibly difficult to detect causal variants or genes of food allergy without access to large studies.

Both genetic and environmental risk factors can assert a collective effect on the development of food allergy. This interplay between genetic and environmental factors, termed, gene-environment interactions, exist when the effect of a genetic variant on disease risk

varies according to the environment (47, 48). The support for gene-environment interaction in food allergy risk is demonstrated by the differential rates of food allergy observed between different populations (e.g Asian and Caucasian population) within the same geographical location (e.g Australia). Although the Asian infants born in Australia were observed to have higher risk of food allergy, the parents of these infants themselves, paradoxically, have low rates of food allergy (49). This supports the notion that living in Australia is not the sole risk factor contributing to the difference in prevalence between the Asian and Caucasian population.

Not taking into account the joint effect of genes and the environment might incorrectly estimate the heritability of complex diseases (50, 51). Ultimately, investigating gene-environment interactions could help with targeted approaches to public health interventions and personalised preventative measures and therapeutics (51). Further, this would also aid in better understanding biological mechanisms and pathways as well as epidemiological observations and associations of food allergy.

1.8 Environmental risk factors for food allergy

Until now, advances in the field have helped characterised key risk factors associated with the development of food allergy. In terms of environmental exposure, these can reflect the macro-environment (e.g. broad climatic and outdoor allergens) or micro-environment (e.g family lifestyle and indoor environment) (52). Additionally, there is also the social environment comprising of factors such as cultural and religious beliefs which can influence familial habits and lifestyle. This can also affect the perceptions on the treatment, symptoms and diagnosis of food allergy. These three broad environment influencers (macro, micro and social) can act in concert to affect development of food allergy.

These largely lifestyle factors which may also be associated with migration, can differ between populations and therefore may explain the observed difference in prevalence of food allergy between the Asian and Caucasian populations (53). These key environmental and/or lifestyle factors are discussed below.

1.8.1 Hygiene hypothesis and microbial diversity

The hygiene hypothesis, first coined by David Strachan showed a protective effect against self-reported hay fever with increasing number of siblings (54). This finding was also replicated in our HealthNuts study where children with older siblings were protective of egg allergy (55). It is thought that shared exposure brought about by common infections through older siblings might have played a role in terms of priming the development of immunoregulatory response. Infrequent infections therefore may contribute to an immune system easily triggered by harmless agents and this presents itself as an allergic reaction. The hypothesis also encompasses several other potential factors such as caesarean section delivery, exposure to domestic pets, antibiotic use, duration of breastfeeding and exposure to microbial products such as endotoxin, all of which could contribute to allergic diseases (56-59).

Naturally, there exists a multitude of differences in terms of microbial exposure between Asian and Caucasian population in their respective countries of origin. This can be in terms of quality of water supply and risk of water-borne gastrointestinal infections (53). There are also potential differences in the microbial composition in the food chain supply potentially as a result of higher use of antibiotics or consumption of unwashed vegetables in Asian countries. Factors such as number of children in a family, overcrowding and exposure to pets, farm animals, and stray animals, as well as variations in overprescribing of antibiotics in each region may also contribute to differences in microbial exposure (53).

Some of these factors have been studied in the HealthNuts cohort with respect to egg allergy, and it was found that children with a pet dog were less likely to develop egg allergy (60). In the same study, there was a lack of association between egg allergy and caesarean section delivery, antibiotic use in infancy, childcare attendance and duration of breast feeding.

However, studies on the association of infections (61, 62) and allergic diseases have been rather inconsistent. A modified “microbial diversity hypothesis” was instead proposed (63), suggesting that improved living standards limit microbial exposure rather than infections, by affecting the type and/or quantity of microbes a child is exposed to. The exposure to microbes from birth is thought to influence the maturation of mucosal immune

system (53). Depending on the composition and timing of exposure to gut microflora, this in turn may modify the infants' gut microbiota in inducing or preventing immunological tolerance, and influence development of allergic diseases. A study involving 24 infants in Canada found that those with older siblings have a reduced microbiota richness and diversity (64). A reduced gut microbiota has previously been associated with allergic diseases (65-67).

1.8.2 Dual allergen hypothesis

The dual allergen hypothesis which stemmed from studies showing co-association between eczema and the development of food allergy was first proposed by Gideon Lack (68). The hypothesis stipulates that a disrupted skin barrier function caused by eczema can promote allergic sensitisation due to low dose epicutaneous exposure to allergens. Without timely introduction of food into the diet, oral tolerance induction does not occur and consequently, food allergy develops (Figure 1.3).

Children with atopic dermatitis are at greatest risk of developing food allergy (69, 70). Our research group demonstrated that infants with eczema were 11 times more likely to develop peanut allergy (95% CI 6.6, 18.6) and 5.8 times more likely to develop egg allergy (95% CI 4.6, 7.4) by 12 months than infants without eczema (71). Among those with eczema, having a father born in East Asia was also a risk factor for food allergy. Additionally, earlier onset and more severe forms of eczema have been shown to increase the risk of developing food allergy. About 50% of infants presenting with severe atopic dermatitis in the first 3 months of life developed food allergy by the age of 1 year (36, 71). Therefore, children with eczema represent a high-risk group that can be targeted for closer monitoring to prevent development of food allergy.

Migration can be associated with changes in skin barrier function and therefore risk of eczema as a risk factor for food allergy in the Asian children in Australia. There may also be differences in infant washing practices in terms of types of soap, water composition and frequency of washing that occur in each country which can have an effect on skin barrier. Moreover, studies have demonstrated that there are differences in the skin immune response (72, 73) as well as epidermal morphology and dermal composition between Asians and Eurasians (74, 75). It is thought that the skin immune response in

Asians is driven by a combination of Th2 and T-helper 17 (Th17) (another T cell effector subset) cytokines while in Europeans the immune response is primarily Th2-mediated (76). In terms of phenotypes of eczema, Asian patients also tend to have well-demarcated, erythematous plaque-like lesions, whereas Caucasian patients typically have ill-defined, flatter erythematous skin lesions. These differences are further supported by another study which found differences in the cytokine profile of Asian and European eczema patients (77).

Studies carried out in mice models provide additional support for the dual allergen hypothesis. Mice with primary skin exposure to peanut or egg protein developed Th2-skewed immune responses evident by antigen-specific IL4 and IgE responses (78, 79). In the presence of a disrupted skin barrier, basophils produced by thymic stromal lymphopoietin (TSLP) mediate a cascade of Th2 responses leading to food allergy (80, 81). Epicutaneously sensitised mice were also found to develop anaphylaxis after intragastric challenge of ovalbumin (82).

Together, this evidence supports the biological plausibility of dual allergen hypothesis in development of food allergy. Additionally, these provide strong evidence that eczema play a contributing factor to differential food allergy risk in Asian versus Caucasian.

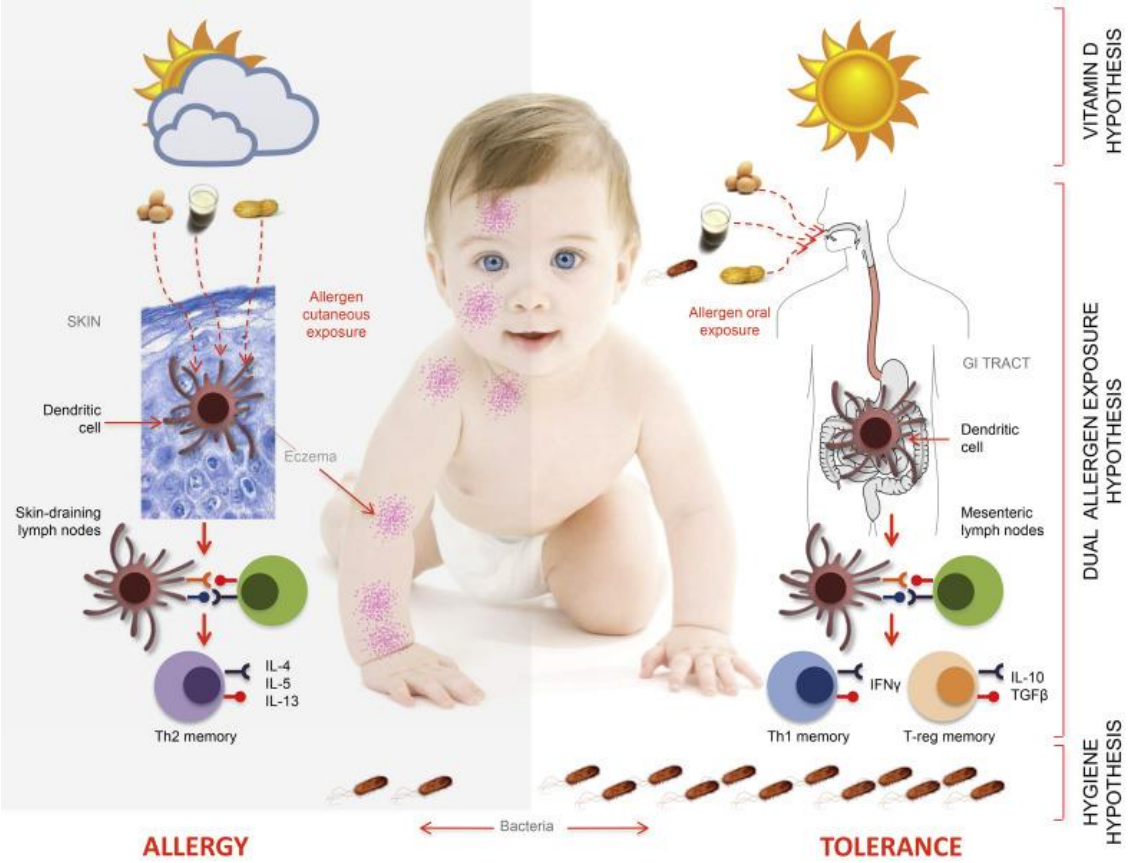

Figure 1.3 The role of hygiene hypothesis, dual allergen hypothesis and vitamin D hypothesis in induction of oral tolerance. GI – Gastrointestinal; T-reg – regulatory T cells. Vitamin D sufficiency, timely introduction of oral exposure to food and microbial diversity are all essential in inducing oral tolerance. In the absence of these protective factors, cutaneous exposure of allergenic food through the skin can promote allergic sensitisation, particularly, for children with eczema due to their disrupted epithelial barrier. Oral tolerance therefore cannot be achieved and food allergy occurs. Figure sourced from du Toit et al (69).

1.8.3 Timing of allergenic food into the diet

In line with the dual allergen hypothesis, observational studies suggest that early introduction of allergenic foods reduces the prevalence of food allergy by induction through the oral route (60, 83, 84). A meta-analysis carried out on existing literature of oral tolerance induction demonstrated that there is ‘‘moderate certainty’’ of evidence that the risk of peanut allergy is reduced with early introduction of peanut between 4 and 11 months of age (relative risk [RR], 0.29; 95% CI, 0.11-0.74; p=0.009) (85). Similarly, moderate-certainty evidence from five trials (1915 participants) showed that early egg introduction at 4 to 6 months reduced the risk of egg allergy ([RR] 0.56; 95% CI, 0.36-0.87; $P = 0.009$). However, findings for fish and early introduction of milk or hydrolysed formula were of ‘‘low certainty’’ and ‘‘no evidence,’’ respectively (85). Conclusions derived from this meta-analysis were based on several clinical trials which were carried out to confirm the observational findings. Primarily, these trials have looked at three main allergenic foods - cow’s milk, peanut and hen’s egg.

1.8.3.1 Cow’s milk introduction

In one prospective study, infants who were introduced to cow’s milk protein formula within the first 14 days of life were significantly less likely to develop cow’s milk allergy compared to infants who had cow’s milk introduced between 105 and 194 days of life (0.05 vs. 1.75%, $p < 0.001$) (84). Further, two other studies showed that delayed introduction of cow’s milk was associated with increased risk of atopy at 2 years (86) and that avoidance of cow’s milk by use of an extensively hydrolysed whey formula did not have a protective effect in infants in developing atopy (primarily eczema) at 12 months of age (87).

1.8.3.2 Peanut introduction

One of the randomised controlled trials assessing peanut introduction is the Learning Early About Peanut (LEAP) allergy study. This study sought to investigate early introduction of allergenic foods as primary and secondary prevention of peanut allergy in high-risk infants (88). Infants were either randomised to avoid any peanut until 60 months of age or to consume peanut products at least 3 times a week (average of 6 g of peanut

protein a week). The authors found that there was a significant reduction in peanut allergic children at 60 months among those who were randomised to consume peanut products regularly compared to the group who avoided peanuts.

Another study, the Enquiring About Tolerance (EAT) randomised controlled trial examined the effectiveness of early introduction of six allergenic foods in the diet of breastfed infants from 4 months of age compared with 'standard' introduction at ~6 months of age in preventing food allergy (89). While the study found lower food allergy in the early introduction group, this was only significant in the analysis based on those who completed randomised treatment originally allocated (per-protocol analysis) and not in the analysis based on original randomised treatment assigned (intention-to-treat analysis).

1.8.3.3 Hen's egg introduction

Our research group has previously demonstrated in a population-based study that at age 4 to 6 months, infants first exposed to cooked egg reduced the risk of egg allergy compared to first introduction as baked egg (OR, 0.2 [95% CI, 0.06-0.71]) (60). Five randomised controlled trials (STEP, STAR, BEAT, HEAP), including EAT (mentioned above), have also investigated the effect of early hen's egg introduction on the development of egg allergy (89-94). Comparisons among these randomised controlled trials is beyond the scope of this thesis but have been comprehensively reviewed by Du Toit et al (95). It was concluded by Du Toit et al (2017) that an important consideration in introduction of food into the diet is the type of egg used – baked, cooked or raw. This could partially explain the mixed findings of association elicited from the various clinical trials in hen's egg introduction.

1.8.3.4 Cultural differences

Cultural dietary preferences and culinary methods might also partially explain the differences in food allergy prevalence between the Asian and Caucasian population. It has been shown that infant feeding practices are influenced by various factors including cultural traditions and beliefs associated with ethnic heritage (96). As one moves to a Western country, there may also be a shift in the types of food introduced to the infants. Foods may also have their allergenicity altered through cooking practices, for example, boiled

versus roasted peanut. Peanuts are commonly boiled or fried in Asian cuisine, which are less allergenic (97) compared with roasted peanuts, which are more common in Western diets (98).

1.8.4 Vitamin D hypothesis

The rise in vitamin D deficiency (99) which coincides with the increase in food allergy prevalence, suggest low vitamin D as a potential risk factor. Indicators of serum vitamin D levels and status, such as latitude mediated through modulation in UV exposure and the skin's ability to produce vitamin D (100, 101), have been associated with the development of food allergy.

UV exposure and vitamin D status is likely to differ between two regions. Food allergy prevalence appears to follow a latitude-dependent gradient in Asia, with higher prevalence in the North Asian countries, such as Japan and South Korea, and lower prevalence in Southeast Asia (7). This latitude-dependent gradient is also observed in Australia, where the region with the lowest ambient UV radiation in the southernmost part of Australia have the highest proportion of population with vitamin D insufficiency and therefore food allergy prevalence, compared to the northernmost Australia (101). Differences in vitamin D status may also be attributed to differences in dietary intake and vitamin D supplementation or fortification programs in each region.

There is a growing body of evidence that lends support to the notion that the relationship between vitamin D insufficiency and food allergy differ by parents' country of birth or migrant status as proxies of ethnicity. Among infants with parents born in Australia, those who were vitamin D insufficient (25-hydroxyvitamin D_3 ($25(OH)D_3$) level of 26 to 50 nmol/L), were more likely to be food allergic (102). However, this association was not observed in infants with non-Australian born parents. In the same study, the relationship was found to be modified by variant rs7041, a proxy marker of vitamin D binding protein levels. There was some evidence that low vitamin D levels were associated with food allergy in infants with the GG genotype of rs7041 compared to those with the GT/TT genotypes (103). Clearly genes are important but they differ in different populations, therefore, it is crucial to recognise that there are population-specific genetic factors as

well as population-specific environmental factors. However, the precise biological mechanisms for the above associations with reference to food allergy is still not yet clear.

Other migration studies in adults have reported immigrants having lower vitamin D levels than the population in the host country. This has been found in the East Asian immigrants in Australia (104) and South Asian immigrants in New Zealand (105). A cross-sectional study in Oslo also showed ethnic differences in vitamin D levels among recently arrived immigrants from Africa and Asia (106). Compared to recent migrants from East Asia whereby only 24% were vitamin D deficient (25(OH)D levels < 50 nmol/L), prevalence of vitamin D deficiency was much higher in migrants from Middle East (81%), South Asia (75%) and South Africa (73%).

Collectively, given the variation in vitamin D deficiency which coincides and have been found to be associated with food allergy, there is strong support for the potential role of vitamin D as a risk factor in the observed food allergy risks in different populations.

1.9 Genetic risk factors for food allergy

While environmental factors play a crucial role in the development or protection from food allergy, the contribution of genetics factors cannot be ruled out. The genetic composition of humans has also been a critical modulator in the development of food allergy. This is supported by studies that have shown family history (i.e heritability) as a strong risk factor for food allergy (107-111).

Heritability estimation are commonly used to determine the degree of influence of genetic factors in complex traits. In a study on 581 nuclear families, total IgE and specific IgE to nine major food allergens were tested (108). In this study, food allergy was defined by a set of criteria obtained via questionnaire interviews and specific IgE cut-offs for positive predictive value for food allergy. Heritability of food-specific IgE was found to be 0.15 to 0.35 indicating that on average, about 15% to 35% of the observed individual differences may be attributable to genetic differences (108). It was also found that food allergy in parents was a major independent predictor of food allergy in their offspring(s). In the same family-based study, a similar association was observed between index child and sibling. However, this finding was contended by Gupta et al who studied 1120 children

with food allergy and concluded that clinical reactivity among siblings (1 in 8) were similar to that in the general population (1 in 12). The study hence, does not support screening siblings before exposure to a food (112). Although these studies illustrate the contribution of genetics in disease risk, a major problem with family-based studies is the inherent environmental risk factors that may act as confounding factors. Studies involving dizygotic twin children alleviates this issue as it can be confidently presumed that the environmental factors for both children are similar and yet, allowed for comparison of genetic influences since dizygotic twins do not have identical genes (113). Conversely, in studies of monozygotic twins, any differences between monozygotic twins are usually attributed to environmental differences, given their identical genes.

To date, there have only been two twin studies that have examined heritability of food allergy or sensitisation in families (113, 114). These studies found that monozygotic twins recorded higher concordance rates for sensitisation to peanut allergen than dizygotic twins. Sicherer et al (2000) examined 58 twin pairs (14 monozygotic twin pairs and 44 dizygotic twin pairs) between the ages of 1 to 58 years old and found that the rate of concordance of peanut allergy for monozygotic twins is 64% while dizygotic twins recorded only 6.8% (113). The heritability estimate for peanut allergy in this study was 82% to 87%, indicating a significant contribution of heredity to the variance in peanut allergy compared to environmental factors. In another twin study of 826 Chinese twin children (472 monozygotic and 354 dizygotic) between the ages of 12 to 28 years old, the heritability estimate for allergen sensitisation to 9 foods and 5 aeroallergens was 51% to 68% (114). The observed disparity in heritability estimate may be attributed to the different outcomes measured, ethnicity and ages of the population in question in each study. Many twin pairs were also discordant for sensitisation for a specific allergen and any allergen (114).

Evidently, the current evidence supports a strong genetic influence in food allergy risk. Complex diseases, such as food allergy, however, are known to be polygenic with many variants each having a small effect size (115, 116). Any single variant therefore would only explain a small proportion of heritability, adding to the challenge of discovering susceptibility genes.

1.9.1 Methods of studying genetic factors

In a quest to identify disease susceptibility genes, studies have used either genome-wide or candidate gene approaches.

Genome-wide association studies (GWAS) are a hypothesis-free driven approach usually carried out using large-scale microarrays that test the entire human genome. While GWAS have been relatively successful in uncovering genetic risks, they tend to focus on common variants with modest effects. Such an approach might miss the contribution of any possible interactions and other genetic variations such as rare variants (variants of low frequency in the population) and copy number variants (variants where the number of copies of a particular gene vary between individuals) (117). On the other hand, candidate gene studies are a hypothesis-driven approach using pre-selected genes of interest based on existing knowledge of biological plausibility or known mechanisms. Given that candidate gene studies require a prior knowledge of the set of genes or single nucleotide polymorphisms (SNPs) to investigate, this approach costs less but may limit the scope to discover novel genes. Candidate gene studies also often yield inconsistent results.

With both genome-wide and candidate gene approaches, common genetic variants also known as SNPs, are identified for an association with a particular trait or disease. In a case-control study design, the presence of a specific sequence or an allele at a particular SNP that is more frequently observed in cases than controls is indicative of a genetic risk factor. Since genes are inherited at birth, presence of these SNPs associated with the disease are therefore causative and are known to affect an individual's susceptibility to disease.

However, due to the co-existence of food allergy with other allergic diseases such as asthma and eczema as well as their shared genetic makeup, identifying SNPs uniquely associated with food allergy can be difficult. This adds to the challenges of studying the genetics of food allergy.

1.9.2 Food allergy genes

The rise of several GWAS in food allergy have led to the identification of several novel genes and polymorphisms associated with food allergy. In particular, the human leukocyte antigen (*HLA*) complex (118-120), serpin family B (*SERPINB*) (121) and chromosome 11 open reading frame 30 (*C11orf30*) have been implicated in several GWAS and meta-analyses (121, 122).

On the other hand, candidate gene studies have broadly focused on genes involved in the immune function, antigen presentation and skin barrier integrity. *FLG* has been the most well studied gene with loss-of-function mutations reported to be a significant risk factor for peanut allergy and pathogenesis of eczema (58, 123-125). These loss-of-function mutations lead to a disrupted skin barrier integrity. In another study, *FLG* mutation was also found to increase the risk of food sensitisation, but not food allergy (126). Other genes investigated include the cluster of differentiation 14 (*CD14*) gene, forkhead box P3 gene (*FOXP3*), interleukin-10 gene (*IL10*), interleukin-13 gene (*IL13*), serine protease inhibitor karzal type 5 gene (*SPINK5*) and signal transducer and activator of transcription 6 gene (*STAT6*) (127-131).

However, most of these studies were carried out in a single homogenous Caucasian population or within one ethnic group. Published reviews on genetic risk factors have also tended to focus on allergy in general or asthma but not food allergy specifically. In order to consolidate the literature on genetic predisposition to food allergy and gain a better understanding of the genetic implications of this disease, this thesis has systematically reviewed the scope of genetic studies in food allergy in Chapter 5.

1.9.3 Ancestry and population structure

A pertinent consideration in genetic studies is ascertainment of study population ancestry which can confound the observed genetic associations. Failure to take population stratification into account in genetic studies may result in spurious association findings, particularly when the population structure is different between the cases and controls. Differences in population structure as reflected by differences in minor allele frequencies (MAFs) between ancestral groups would confound the association between the genotype

and disease outcome. Any observed associations may be due to differences in ancestry that are unrelated to the disease risk.

Such false positive associations due to confounding by population structure are well-documented. To quote a hypothetical example, a genetic study set out to investigate association between using chopsticks and *HLA* in a population in San Francisco may find a positive association between allele HLA-A1 and ability to use chopsticks in the East Asian population in San Francisco. It is unlikely that there exist an "immunological basis for dexterity" of using chopsticks. Rather, the association was simply due to a higher frequency of HLA-A1 among the East Asian population than the Caucasian population in San Francisco (132). The observed association would therefore be lost after adjusting for population structure.

One way of controlling for population structure is by using multidimensional scaling (MDS) and principal components (PC) analysis estimated from a set of markers which are also known as ancestry informative markers (AIMs). These AIMs have distinct MAFs between ancestral populations and are incorporated into regression models as covariates to account for ancestry. Data obtained from an AIMs panel would provide a more reliable measure of ancestry compared to available data on self-reported ancestry which may be inaccurate due to vague responses or overly simplistic answers.

1.9.4 Mismatch hypothesis

This thesis has established that there are distinctions in environmental factors that may explain the difference in food allergy prevalence between East Asians and Caucasians in Australia. Genetically, one hypothesis that may potentially explain this finding is the mismatch hypothesis. At the heart of this hypothesis is the notion that human evolution occurred in conditions different to that of the current western environment and consequently may affect an individual's biology and adaptations to new environment. Commonly known as the 'Out of Africa' theory, the human species expanded beyond Africa to inhabit the rest of the world (133). This exposure to widely differing ecological environments upon migration out of Africa, may have contributed to human diversification and influenced genetic differentiation that can affect vulnerability to some diseases (134).

Notably, human immune systems may have genetically developed to adapt to the different climatic environments (135, 136).

In the context of food allergy, it is postulated that evolutionary pressures have selectively conditioned populations residing in tropical environments towards an inflammatory Th2-skewed immune response (135). The Asian genome may have evolved under the context of higher tropical pathogen exposure that drove selection of particular alleles in the IgE system that are unique to populations living in that geographical region. It is known that pathogens such as helminths evoke strong IgE-mediated immune response and thrive in heat and humidity (137). In addition, the IgE aspect of human immunity is thought to have evolved as a host defence mechanism against parasites and venoms produced by certain arthropods. Therefore, populations living under a high pathogenic load may be enriched for certain genotypes that favour enhanced IgE-mediated immunity. Lack of adequate counter-regulatory control of IgE-mediated inflammation (for example in sub-tropical regions) could also predispose to allergic diseases (134, 135). In that vein, gene variants with pro-Th2 functions are predicted to be more common in those with long-term tropical ancestry (Asians) than those of temperate origin (Australians).

The mismatch hypothesis suggests our evolved capacities are mismatched to modern environments and certain genes that were favourable in traditional environments become risk factors for disease (138). An example of such selection pressure is the finding that populations protected against dehydration in hotter areas in the past may have descendants who are susceptible to hypertension (139). Sodium retention is thought to play a role in heat tolerance and is therefore greatest in hot and humid environment. With the change to colder climate that the descendants of these heat adapted populations may face, their sodium retention capability would be maladaptive. The same study also found a higher frequency of heat-adapted alleles in populations of hot and wet climates compared to populations in a cold and dry climate.

In another key example of evolutionary selection of the immune system, a study of HLA allelic diversity and pathogen richness in 535 populations found a correlation between geographical differences and HLA allelic diversity. This genetic differentiation is positively correlated with pathogen richness at loci of *HLA-A* and *HLA-B* (140). This provides strong support that HLA genes have been subjected to evolutionary pressures in order to

provide immune protection in pathogen-rich environments. This finding has significant implications and applicability in food allergy as several GWAS have reported associations between variants of *HLA* and risk of food allergy (118-120). Fumagalli et al extended support for the mismatch hypothesis by showing that pathogen richness are correlated with SNP diversity in asthma and allergy genes (141).

Other studies carried out in Th2-related polymorphisms and diseases have also elucidated similar findings, where differential frequencies are observed in different populations. One such example is the -589T allele of *IL4*, which was associated with increased serum IgE concentrations (142). The -589T allele is found to vary by ethnicity, being more common in African Americans with a MAF of 0.522 compared to white Americans with a MAF of 0.183 (142). Another classic example is *FLG*, which has been shown to be a risk factor for eczema and vary considerably among populations. In an Ireland population, two *FLG* mutations (R501X and 2282del4) accounted for 80% of the risk alleles among those with eczema. Conversely, those with eczema in Singapore had 16 recurrent mutations contributing only 1 to 24% of total risk (143). While these illustrations did find a differential MAF between different populations, it remains to be seen if they are a result of historical selective adaptations. Nonetheless, this reflects a need for future studies to address population stratification, if not tailor and limit studies to specific populations.

1.10 Distinguishing race, ethnicity, ancestry and genetic background

Prior to engaging in an in-depth discussion of population effects of food allergy in the remaining sections of this thesis, it is imperative to distinguish between the terms race, ethnicity and ancestry. These terms have commonly been used interchangeably given the lack of a mutually agreed definitions for each term. Collectively, all of these terms encompass aspects of an individual's cultural, social, historical, religious, ancestral and geographical origins. Racial categorisation have a tendency to be based on skin pigmentation, particularly in the United States (144-146). On the other hand, ethnicity is often referred to as a self-defined identity based on geographical, social, cultural and/or religious beliefs (145).

In defining ethnicity in Australia, the Australian Bureau of Statistics adopts the Australian Standard Classification of Cultural and Ethnic Groups (ASCCEG) that classifies ethnicity according to nine broad groups "Oceanian, North-West European, Southern and Eastern European, North African and Middle Eastern, South-East Asian, North-East Asian, Southern and Central Asian, Peoples of the Americas, Sub-saharan African" (147). According to ASCCEG, ethnicity is described as "a self-perceived group identification approach, and an approach that is more historically determined" (147). In agreement with Risch et al (2002), this would more often than not include cultural, socioeconomic, religious and political rather than genetic ancestry (144).

Epidemiological studies often collect ethnicity information as part of their study. However, conflicting views exist regarding the use of race, ethnicity and ancestry data in medical studies – with some arguing that race is a purely social concept with no biological relevance (148) (149, 150). Contrary to this school of thought, one area in which racial, ethnic and ancestral categories are important is in genetics research to control for confounding by population structure as discussed in Section 1.9.3.

A purely biological construct, ancestry reflects human evolution after the major migration periods of early humans. Ancestry then, refers to the lineage from which an individual's genes can be traced back to. The International HapMap project is an example of a genetic study which defined human ancestral groupings as reference populations (151). The HapMap project was designed to identify variants across the genome and their frequencies by using DNA samples from populations with ancestry from parts of Africa, Asia and Europe (151). Since its conception, HapMap has been one of the most comprehensive resource and research tool to investigate genetic factors of diseases.

With these in mind, our research group has previously demonstrated that self-reported ethnicity correlated well with genetically inferred ancestry (130). For this reason, this thesis used the terms ethnicity when referring to data collected from questionnaires (self-reported) and ancestry when referring to genetically inferred ancestry. A detailed description of this correlation between genetically inferred ancestry and self-reported ethnicity is provided in later sections (Chapters 2, 6 and 7).

1.11 Rationale for study

There is strong epidemiological evidence that children of Asian descent are genetically predisposed to food allergy. Absence of potentially protective environmental factors upon migration of Asian parents to Australia, appear to unmask this predisposition in their children. This is reflected by the finding that the Asian population in Australia is more vulnerable to food allergy compared to the Caucasian population, despite food allergies in Asia being relatively low. The two population groups (Asian and Caucasian) originate from different environments that differ both in terms of climate and lifestyle. The abrupt change in composition of environment and allergen exposure, as well as gradual changes in lifestyle and habits, associated with migration may be the underlying cause of the rapid rise in food allergy observed in Asians living in Melbourne. Therefore, this cohort of Asian infants in Melbourne presents a unique and rare opportunity to explore the pathways of food allergy among different ethnic populations in the same geographical location.

Additionally, despite rapidly growing literature on food allergy, studies tend to focus on the Caucasian population. The Asian population is increasingly becoming an important population to study, as this population appear to be particularly vulnerable to food allergy not just in Australia but around the world. The increased risk of food allergy in Asian children in Australia is largely understudied and research into such studies will make important contributions to our understanding of the observed differences in prevalence.

This research area is also particularly pertinent now as there has been an influx of Asian-born migrants into Australia predominantly from China and India in recent years (152). According to the Australian Bureau Statistics, 29% of Australia's resident population was born overseas, with a majority of those from Asia (153). Despite this, there have been few investigations into the health conditions of the Asian population at the national level. Better understanding of food allergy mechanisms and underlying causes of this phenomenon in the Asian population is crucial in minimising the adverse social and financial implications on the healthcare system in the long run.

With that in mind, this thesis sought to identify environmental and genetic risk factors contributing to the increased food allergy risk in children with East Asian-born parents

compared to children with Caucasian-born parents. In doing so, this thesis will expand the limited knowledge on risk factors for food allergy in Asian populations residing in Australia. Specific research questions addressed in each chapter of this thesis are detailed below.

1.12 Research questions

The work carried out within this thesis were based on data and samples obtained from a well-characterised longitudinal, population-based study of food allergy in Melbourne, Australia, known as the HealthNuts study. Additionally, a birth cohort study in Singapore, known as the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) was also used to compare the prevalence and risk factors of food allergy in East Asians living in Singapore and East Asians living in Melbourne, Australia. Using these two studies, this thesis explored the following questions:

1.12.1 Exploring environmental risk factors

Chapter 3 – HealthNuts study

1. What is the prevalence of asthma, allergic rhinitis, and aeroallergen sensitisation at age 6 in the infants with Asian-born parents living in Australia compared to those with Caucasian-born parents living in Australia?
2. Is infant food allergy and eczema status at age 1 year associated with childhood asthma and allergic rhinitis at age 6 years in the infants with Asian-born parents living in Australia?
3. How do these associations compare to infants with Caucasian-born parents living in Australia?

Chapter 4 – HealthNuts and GUSTO studies

1. Is there a difference in the prevalence of food allergy between infants with East Asian parents living in Singapore and those living in Australia?
2. What are the risk factors for food allergy in each of the East Asian populations?
3. Does the prevalence of these risk factors differ between Singapore and Melbourne, Australia?

1.12.2 Exploring genetic risk factors

Chapter 5 – Systematic review of published literature

1. Based on current literature, which genes have been studied in relation to food allergy and what is the evidence for an association between genetic polymorphisms and food allergy?

Chapter 6 – HealthNuts study

1. Which helminth-related and/or allergy SNPs are suitable candidates for genotyping in the HealthNuts biological specimens, for future investigation of their association with food allergy?

Chapter 7 – HealthNuts study

1. Are helminth-related SNPs associated with food allergy in the East Asian population living in Australia?
2. Are these SNPs also associated with food allergy in the Caucasian population living in Australia?

1.13 Overview of thesis

The remaining sections of this thesis is organised as follows:

In the next section, **Chapter 2** describes the overall methods and definitions used in analyses and experiments carried out in this thesis.

Investigation of environmental risk factors for food allergy are presented in Chapters 3 and 4. In **Chapter 3,** this thesis presents the analyses on the risk of asthma and allergic rhinitis in early childhood among infants with East Asian-born and Caucasian-born parents. **Chapter 4** compares the prevalence and risk factors for food allergy between Asians living in Singapore and Asians living in Melbourne.

My investigation on the genetic risk factors for food allergy are discussed in Chapters 5 to 7. **Chapter 5** describes a systematic review of the current literature on genetic risk factors for food allergy while **Chapter 6** outlines the methods adopted in selection of

candidate genes and SNPs for genotyping. Results obtained from genotyping are reported in **Chapter 7**.

Finally, **Chapter 8** concludes with a discussion of the results along with suggestions for future research.

Chapter 2 Overview of methodology

This thesis used data and samples obtained from a well-characterised longitudinal study of food allergy. The longitudinal population-based study known as the HealthNuts study, recruited 12-month-old infants at immunisation centres across Melbourne, Australia. Data and biological specimens collected from the HealthNuts study were used for analyses throughout this thesis. This thesis also used data collected from a birth cohort in Singapore, known as the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) to compare prevalence and risk factors for food allergy in East Asians from different geographical regions. The methods and results of this investigation using the GUSTO study is described in Chapter 4.

This chapter aims to present an overview of the methods and recruitment process of the HealthNuts study. Further chapter-specific methods are provided in each of the respective results chapters. Figure 2.1 below illustrates the study pipeline of this thesis.

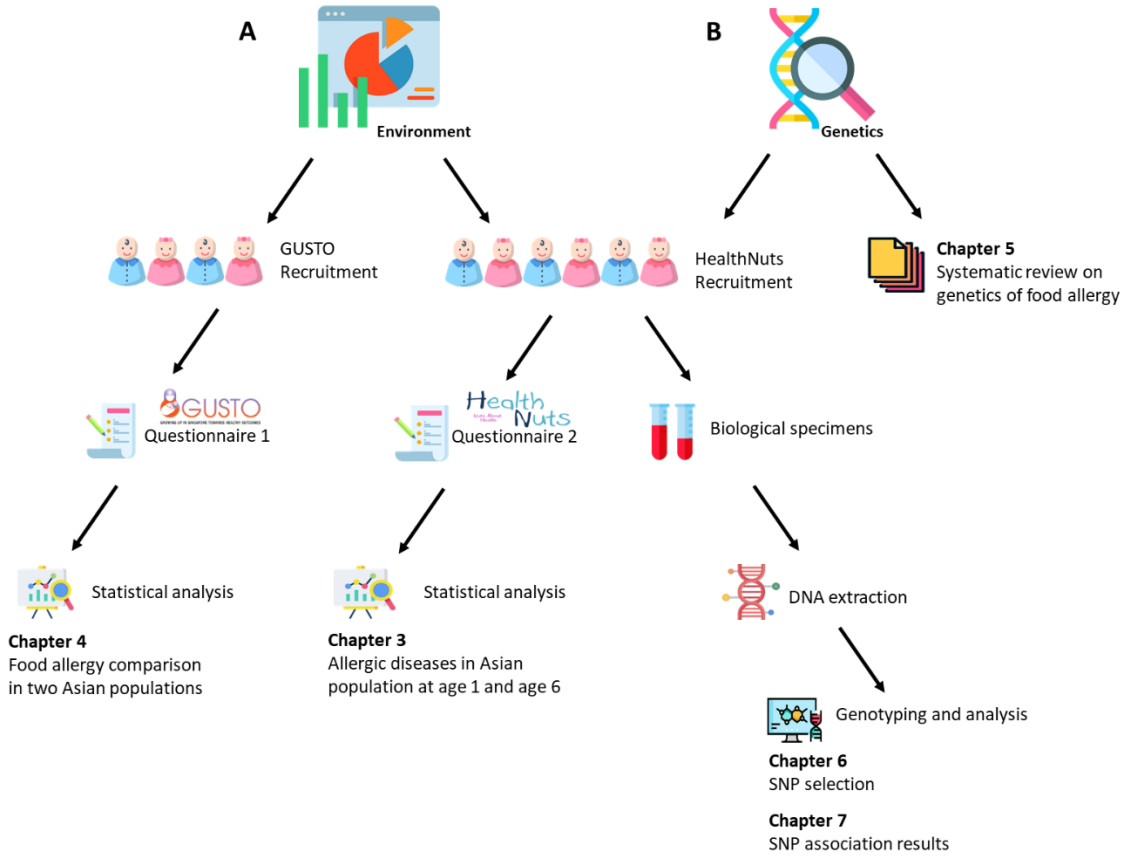

Figure 2.1 Study pipeline of projects involved in this thesis. SNP – Single Nucleotide Polymorphism. A) In exploring environmental risk factors, the HealthNuts cohort and Growing Up in Singapore Towards healthy Outcomes (GUSTO) cohort were used. Chapters that addressed the environmental risk factors for food allergy are in Chapters 3 and 4. B) Exploration of genetic risk factors was carried out using the HealthNuts study and a systematic review of current literature. Results for the genetic aspect were reported in Chapters 5 to 7.

2.1 Study cohort

The study population investigated in this thesis were participants from the HealthNuts study. HealthNuts is a population-based longitudinal study on food allergy in Melbourne, Australia. The overall aim of the initial HealthNuts study was to obtain the prevalence and identify risk factors for food allergy in 12-month-old infants residing in Melbourne, Australia. The 12-month-old infants were chosen as it was believed that most infants would not have ingested peanut at this age (154). Infants were recruited through council run immunisation sessions across Melbourne. In general, on the day of recruitment at the

immunisation sessions (12 months), infants underwent SPT to a panel of allergens and parents completed a health questionnaire pertaining to their children's health. For infants with a positive SPT, they were followed up at the HealthNuts allergy clinic at Royal Children's Hospital for an open OFC. Open OFCs were used instead of double-blind placebo controlled food challenge as this was deemed to be the gold standard for diagnosis in infants, in line with the PRACTALL consensus report (155). Given their young age, infants were unlikely to elicit false positive symptoms of reactivity brought about by anxiety compared to older children or adults.

With a high participation rate (73%) and high attendance rate (84%) at the HealthNuts allergy clinic, the potential effects of selection bias related to the higher likelihood of participation by those at a higher risk of allergy were minimised. Summary statistics of the HealthNuts cohort were comparable to that of the Perinatal Data Collection Unit, which collects detailed data on all Victorian births (156). Apart from a slightly higher maternal age and socioeconomic status, the HealthNuts cohort was largely similar to the census of infants born in Victoria, suggesting that it was broadly representative of the population.

A key strength of the HealthNuts study in addressing the research questions outlined in the previous chapter, is the availability of objective measures and outcomes of allergic diseases such as SPT and, OFC which will be described further in the following sections.

2.1.1 Recruitment and clinical assessments at age 1 year

2.1.1.1 Recruitment

Parents of infants between 11 – 16 months of age who were attending their 12-month immunisation sessions were approached to participate in the study between 2007 and 2011. In total 5,276 infants were recruited. These infants underwent SPT during the 15-minute post-immunisation waiting period and their parents were given questionnaires to fill in (Appendix 1). Those who declined to participate were asked to complete a non-participant questionnaire which collected information such as reasons for declining to participate, food allergy history, eczema history and home postcode (used as a measure

of socioeconomic status) (Appendix 2). This information allowed us to identify any participation bias on the basis of history of allergy in the child or siblings or socioeconomic status. Infants with severe eczema or with a history of anaphylaxis were not tested in the community but were invited to attend the HealthNuts allergy clinic at the Royal Children's Hospital where assessments were carried out under medical supervision.

2.1.1.2 Clinical assessment at immunisation session

At the 12-months immunisation sessions, all infants underwent SPT to test for sensitisation to egg white, peanut and sesame (ALK, Madrid, Spain), positive control (histamine 10mg/mL) and negative control (saline). Shrimp allergen was also included in the SPT of the first 2500 infants. This was then replaced with cow's milk in the remaining 2500 infants in October 2009. SPT to shellfish was replaced with cow's milk as the study had obtained confirmation on the low sensitisation rates of shellfish. Cow's milk was originally excluded from the SPT panel as we were unable to obtain approval from the ethics committee to include it in the panel. This was primarily due to growing concerns from the committee and anxiety in the community that a high prevalence of sensitisation would be observed in infants already tolerating cow's milk. We were able to reverse the decision when a pilot study found low cow's milk sensitisation rates at age 12 months.

SPT was done on the infant's upper back using lancets and the width and height of wheal were recorded after 15 minutes. Infants found to have SPT wheal size of ≥ 1 mm greater than the negative control to any of the four foods were invited to the clinic at the HealthNuts allergy clinic at the Royal Children's Hospital for a repeat SPT and OFC to ascertain their food allergy status. The threshold of 1 mm was chosen as a screening tool at immunisation sessions to capture all possible food allergy cases but this threshold was not used to define food allergy status in our analyses.

2.1.1.3 Clinical assessments at HealthNuts allergy clinic

A total of 1129 infants attended the HealthNuts allergy clinic at 12 months. At the HealthNuts allergy clinic, a research nurse and specialist allergist noted down the clinical history of the infants. The nurse performing the challenge and the supervising physician were blinded to the infant's skin prick test results.

A repeat SPT was carried out at the HealthNuts allergy clinic which included the initial panel of allergens (egg, peanut, sesame, cow's milk/shellfish) as well as hazelnut, almond, cashew, soy, wheat and house dust mite (*Dermatophagoides pteronyssinus*). All allergens were from ALK (Madrid, Spain) except for cashew (Hollister-Stier Laboratories, USA).

Infants who were sensitised to egg, peanut or sesame underwent OFCs to each food that the infants were sensitised to. An exception was made if the infant was currently avoiding the food and had shown objective signs of acute allergic reaction to particular food within one month prior to a positive skin prick test to egg, or two months prior to a positive skin prick test in the case of peanut or sesame. The OFCs occurred at least one week apart and were done according to the protocol used by the Department of Allergy at the Royal Children's Hospital, Melbourne (156, 157). The relevant allergenic food was administered in increasing doses in intervals of 15-20 minutes, beginning with a smear or drop inside the lip until reaching the final dose or a reaction occurred. As a follow-up to the OFCs, research nurses would phone all food challenged participants the next day to capture late reactions. Objective criteria as listed below, were used to define a positive OFC and these were described in detail in Koplin et al (157).

Negative OFC: An OFC is classified as negative if the infant is tolerating the maximum dosage of the food with no reported reactions. For parents whose infants had a negative OFC, they were asked to serve the challenge food daily at home over a period of seven days while completing a daily symptoms questionnaire.

Positive OFC: An OFC is classified as positive if one of the following criteria are met:

i) One or more of the following symptoms during OFC to egg, peanut or sesame:
   - Three or more concurrent, noncontact hives/urticaria lasting at least five minutes and/or
   - Perioral/periorbital angioedema and/or
   - Vomiting excluding "gag" and/or
   - Anaphylaxis (evidence of circulatory or respiratory compromise such as wheeze, cough, change in quality of cry, or respiratory distress) within two

hours of the last dose within the food challenge, as defined by the Australian Society of Clinical Allergy and Immunology **or/and**

ii) Any of the above reactions occurred within two hours of the last dose within the food challenge or within two hours of ingestion of the food on days two to seven of the challenge at home

Inconclusive OFC: Infants who refused to eat the challenge food was deemed as inconclusive. Those with an inconclusive challenge were invited for a repeated challenge.

2.1.1.4 Questionnaire

Information collected in the self-administered questionnaire (Appendix 1) included general information about the child (e.g. mode of delivery, gestational age, childcare attendance), child's diet (e.g. introduction of allergenic food, formula feeding, duration of breastfeeding), family history of allergy (e.g. eczema, asthma, food allergy), maternal diet and medications during pregnancy, child's health during the first year of life (e.g bronchiolitis, antibiotics use, wheezing) and child's family (e.g any smoking, number of siblings, pet ownership).

2.1.2 Follow-up studies

The 12-month-old infants were followed up at ages 2, 4 and 6 years. An overview outlining the follow-up studies is illustrated in Figure 2.2. The aim of the age 2 follow-up was to determine the natural history of egg and baked egg allergy. Meanwhile, age 4 years and 6 years follow-up studies sought to explore the rate of tolerance and persistence of food sensitisation and food allergy.

Analyses in this thesis used data collected at 12 months and age 6 years follow-up. Follow-up data at age 6 years instead of other time points was chosen because asthma diagnosis is generally only considered possible after 5 years of age. Age 6 years was also chosen to allow assessment of the range of allergic outcomes. Comorbidities associated with food allergy such as asthma, allergic rhinitis and aeroallergen sensitisation often develop after infancy, making it impossible to assess these conditions at age 1 year. Further, SPT to aeroallergen was only carried out at age 6 years.

The follow-up study at age 2 years was a nested study within HealthNuts. Infants who were followed up at age 2 years were those with challenge-confirmed raw egg allergy at age 1 year who were subsequently offered baked egg OFCs. These infants were followed up at age 2 years, with repeat OFCs to raw egg to investigate early resolution of egg allergy. Further details on recruitment and analyses done in this follow-up study can be found in a study carried out by Peters et al (24).

All children recruited at 12 months were eligible to participate at the follow-up studies at ages 4 and 6 years. These follow-ups serve to explore persistence and resolution of food allergy and risk factors associated with food allergy. Further, it allows for the study of inter-relationships with other allergic diseases such as asthma, allergic rhinitis and eczema. Details on recruitment and prevalence of food allergy in the age 4 year follow-up study has been described by Peters et al (158).

At age 6 years, all participants in the cohort were offered a visit to the HealthNuts allergy clinic at the Royal Children's Hospital to assess food allergy, eczema, asthma and allergic rhinitis. Those who were unable to attend a clinic visit in the hospital were offered a home visit. If parents were still unable to attend, they were asked to complete the questionnaire which were mailed to them. The following sections below will focus on the study design at 6 years as the data at age 6 years were also used in subsequent analyses carried out in this thesis.

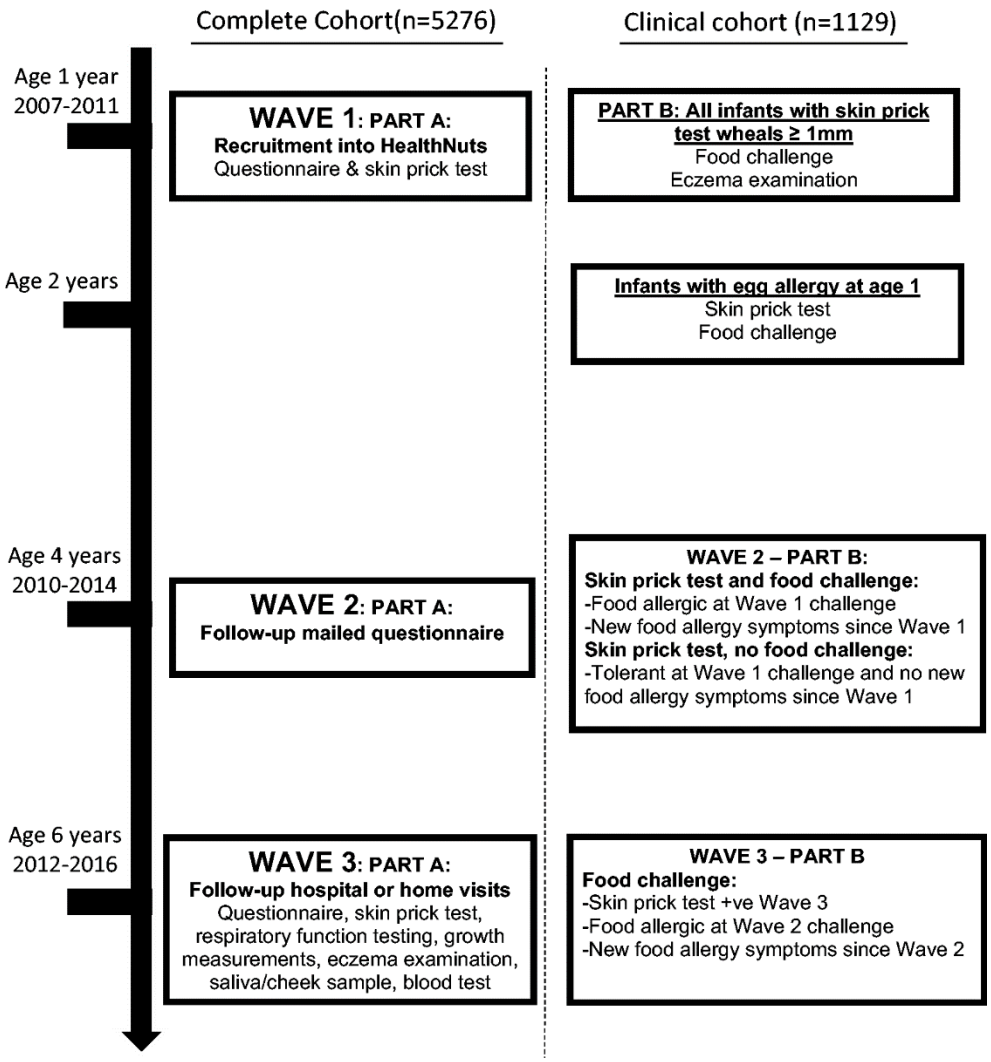

Figure 2.2 Summary of recruitment and assessments in each HealthNuts follow-up study. At age 1 year, infants attending council-run immunisation sessions were eligible for recruitment into the study (complete cohort). Skin prick tests were carried out at immunisation centres and parents were invited to the HealthNuts allergy clinic if infants had a skin prick test wheal size of more than 1 mm (clinical cohort) to any of the food allergen tested. At age 2 years, only those with egg allergy and baked egg allergy were followed up. At ages 4 and 6 years, the complete cohort at age 1 year were contacted for a follow-up study. Sourced from Koplin et al (159).

2.1.3 Recruitment and clinical assessments at age 6 years

2.1.3.1 Recruitment

All parents in the study were mailed an information statement, consent form and a questionnaire two weeks before their child's 6th birthday. The consent form must be completed to take part in the clinical assessment.

2.1.3.2 Questionnaire

Parents were asked to complete a written questionnaire or an online version via Survey Monkey. This questionnaire covered similar questions to that obtained at 12 months (e.g. history of child and family allergic disease, lifestyle, quality of life) in addition to extra questions that were collected to assess the resolution of food allergy and development of other allergic diseases in the cohort. Questions pertaining to other allergic diseases such as asthma and allergic rhinitis were also included based on validated International Study of Asthma and Allergies in Childhood (ISAAC) questions (160). We also collected information on parents' and grandparents' self-reported ethnicity.

The age 6 years questionnaire is provided in Appendix 3. Definitions of specific questions used in analyses is provided in each chapter and below in Section 2.1.4.

2.1.3.3 Clinical assessments

Participants were first invited to attend clinical assessments at the HealthNuts allergy clinic or offered home visits if they were unable to attend the clinic. The same clinical assessments, comprising of skin prick tests, eczema examination and venepuncture blood collection, were also carried out at home visits. At home visits, blood samples were collected by a finger prick spotted onto Guthrie cards.

Compared to the panel of allergens tested at 12 months, participants undergo SPT to a wider range of food allergens and aeroallergens at age 6 years. Ten of the most common food allergens - peanut, sesame, soy, almond, cashew, hazelnut, egg white, shellfish, cow's milk and wheat and eight of the most common aeroallergens - house dust mite, cat,

dog, rye grass, bermuda grass, birchmix and molds (*Alternaria* and *Cladasporum*) were used.

At the end of the clinical assessment, parents of children with a positive skin prick test result to foods were invited to make an appointment for an OFC. Those with new-onset symptoms of food allergy as well as those with food allergy at age 4 follow-up or since last seen were also invited to the HealthNuts allergy clinic for OFCs. Description of OFCs that were carried out has been detailed earlier in Section 2.1.1.3.

2.1.4 Definitions of outcomes

Definitions of variables that were used across all chapters (food allergy outcome at age 1 year and ancestry background) are provided in this section. Definitions for environmental exposure and outcomes used for analyses that are unique to each chapter are provided in the relevant chapters (Chapters 3-7).

Food allergy: Infants were classified as having food allergy if they had either a

i) positive SPT defined as SPT wheal size is ≥ 2 mm to at least one of the following (egg, peanut, sesame) on the day of OFC in the context of a negative saline control and positive histamine control **OR** positive CAP-FEIA (0.35 kU/L) to the food in question measured during the clinic assessment **AND**
ii) positive OFC as defined earlier.

Ancestry: Ancestry definition was based on parent's country of birth. Parent's country of birth were obtained for both parents. This information was classified into the following categories: Australia, UK/Britain, Europe, Mid East, Africa, South America, North America, Oceania, South Asia and Far East.

A categorical variable was created which defined parents' country of birth into the following three categories: Both parents born in Australia, UK or Europe, one parent born in East Asia (the other born in Australia), both parents born in East Asia.

Infants were classified as ***East Asians*** if one or both of their parents are born in North East Asia region (China, Hong Kong, Japan, Taiwan, Macau, North Korea, South Korea)

and South East Asia region (Vietnam, Philippines, Singapore, Thailand, Indonesia, Malaysia, Cambodia, Laos). Caucasians are children with parents born in Australia, New Zealand, UK, Europe or North America. These countries were grouped based on the Standard Australian Classification of Countries (SACC) which were developed to be relevant to Australia's multicultural society for use in analysing Australian-based country of origin data (161). Groups in the SACC comprise geographically proximate countries which have broadly similar social, cultural, economic and political characteristics. Figure 2.3 shows a schematic representation of countries classified as East Asia.

Caucasians are made up of children with both parents born in Australia, UK or Europe for all analyses.

Those with parents not classified as East Asians or Caucasians were excluded from all analyses.

The above classification of ethnicity has been shown to be a good marker of ancestry, as shown by a genetic panel analysis in a sample of 534 infants across different studies (N=108 from the HealthNuts study). The genetic panel, also known as AIM panel (see section 1.9.3) are made up of variants with frequencies that vary substantially between different populations (e.g Caucasians, Asians and Africans). The analysis showed that the genetically inferred ancestry correlated well with self-identified ethnicity and publicly available genetic data on Caucasian and Asian populations (131).

The parent's country of birth as proxy of ancestry was also correlated with collected information on grandparent's ethnicity. 87% of children classified in the East Asian group had at least one set of grandparents reporting Asian as their ethnicity whereas 80% of those in the Caucasian group had both sets of grandparents reporting as Caucasian. Grandparents' ethnicity information were categorised as either Caucasian, Asian, African, Aboriginal/Torres Strait Islander, Middle Eastern or Other.

Figure 2.3 Infants with parents born in the countries shaded in black are classified as East Asians according to the Standard Australian Classification of Countries (SACC) which were developed to be relevant to Australia's multicultural society for use in analysing Australian-based country of origin data (161).

2.1.5 Ethics

Ethics approval for all HealthNuts follow-up was obtained from the Human Research Ethics Committee (HREC) of the Victorian State Government Office for Children (reference no. CDF/07/492) and Department of Human Services (reference no. 10/07) as well as the Royal Children's Hospital HREC (reference no. 27047 and 32294). Parents gave written consent on behalf of their child for participation in the study.

2.2 Collection of biological samples

Following the completion of OFC or clinical assessments at the hospital or home visits, participants were asked for consent to collect blood samples. I was responsible for the

processing of blood samples that were collected during the first year of my PhD (April 2015 – July 2016).

Venepuncture blood of 7-14 mL was collected either by research nurses at the HealthNuts allergy clinic or by nurses at the pathology clinic of the Royal Children's Hospital. For those who opted for home assessments at age 6 years, blood was obtained via a finger prick onto a Guthrie card.

In addition to the venepuncture blood collected during assessments and OFC, parents were also approached to obtain consent to access their child's newborn screening cards (NBS cards). These NBS cards were collected as part of a screening program to detect any rare, serious but treatable medical conditions in newborn babies. The newborn's heel was pricked and blood spotted onto the NBS cards. We were interested in obtaining access to NBS cards to investigate whether vitamin D levels in early life affects the risk of food allergy. These NBS cards were also used as a source to extract DNA for genetic studies. In total, 2700 parents gave consent to accessing their child's NBS cards. Only samples of children whose parents consented to the use of their child's NBS cards for measuring vitamin D levels and future ethically approved research were used for genetic studies carried out in this thesis. The rest consented to the use of NBS cards only for measuring vitamin D levels.

2.2.1 Negative controls

In order to obtain biological samples from negative controls (negative SPT and negative OFC), a subgroup of participants who attended the HealthNuts allergy clinic were asked to participate in OFC. These participants had a SPT wheal size of 0 mm to all food tested and underwent OFC to either peanut or egg using the same protocol established earlier in Sections 2.1.1.2 and 2.1.1.3.

2.3 Processing of biological samples

2.3.1 Venepuncture blood

Up to 14 mL venepuncture blood was collected into sodium heparin tubes (BD, USA) and processed within 2 hours of blood collection to maximise cell viability. Plasma was removed through centrifugation before being stored at -80˚C for later analysis of biomarkers such as cytokines and chemokines. If not processed immediately, 10 mL of transport media containing 10 IU/mL preservative free heparin in Gibco® RPMI-1640 (Invitrogen, USA) and 10% heat inactivated fetal calf serum were added to the remaining blood sample. Peripheral blood mononuclear cells and granulocytes were separated using a standard ficoll procedure (Appendix 4). The cells were cooled slowly to -80˚C, before being transferred to liquid nitrogen storage for long term storage.

Meanwhile, the tube of aspirated granulocytes was filled with 1 x Boyles solution (diluted from 10 x Boyles stock solution) and mixed by frequent inversion at room temperature until lysis of red blood cells has occurred (opaque red liquid turned transparent red). Upon lysis, the tube was centrifuged at 805 *g* for 10 minutes. Supernatant was removed and 10 mL of phosphate buffered saline was added to resuspend and rehydrate the cells. The tube was centrifuged again at 350 *g* for 10 minutes and supernatant discarded. Freezing mix (750 μL) was added dropwise into the tube and stored in -80˚C for future DNA extractions.

2.3.2 Newborn screening cards

All NBS cards were stored in a repository held by Victorian Clinical Genetics Services (VCGS). A previous PhD student, Rosita Zakariaeeabkoo and a volunteer were able to recover 2670 of 2700 NBS cards of infants whose parents consented to the access. Ten 3 mm punches from each NBS card were collected into a microcentrifuge tube for storage at -80 degrees.

2.4 DNA extractions

DNA required for genotyping had to be extracted from either venepuncture blood (or Guthrie cards if collected from home visits) or NBS cards. Given that a participant may

provide more than one biological specimen, participants' DNA was extracted from venepuncture blood where available and if not available, DNA was then extracted from NBS cards or Guthrie cards.

2.4.1 DNA extracted from venepuncture blood

Granulocytes obtained from the whole blood were used to extract DNA. Genomic DNA from granulocytes was extracted using QIAGEN® Flexigene DNA kit (QIAGEN®, Germany) according to manufacturer's protocol for a 100 – 500 μL buffy coat detailed in the FlexiGene DNA Handbook (February 2003).

Protocol for 200 μL buffy coat was followed as this was judged to be the most closely related sample type to granulocytes. Prior to starting, the total volume of granulocytes to be processed was calculated and a proprietary buffer FG2/QIAGEN Protease mixture was prepared first according to the proportions listed in Table 2.1. Briefly, 500 μL of Buffer FG1 was added to 200 μL of the granulocytes obtained from the granulocyte isolation method described in Section 2.3.1. This allows lysis of cells to occur and thereafter the mixture was centrifuged for 20 seconds at 10,000 *g*. Supernatant was discarded and 200 μL of a mixture of buffer FG2 and QIAGEN Protease (800μL:10μL) was added to the tube and vortexed until the pellet completely homogenised. The sample was centrifuged again followed by incubation at 65°C for 10 minutes. 200 μL of 100% isopropanol (Sigma-Aldrich, USA) was added to the sample and mixed by inversion until the DNA precipitate became visible. The sample was centrifuged for 3 minutes at 10,000 *g* and supernatant discarded before washing with 200 μL of 70% ethanol. The remaining DNA pellet was air-dried and eluted in 200 μL of milliQ water. DNA was then dissolved by incubation for 30 minutes at 65°C.

Table 2.1. Volumes of Buffer FG2 and QIAGEN Protease required for different batch volumes

Total volume of buffy coat in batch (µL)	**100**	**300**	**500**	**1000**	**3000**	**5000**	**6000**
Volume of Buffer FG2 (µL)	100	300	500	1000	3000	5000	6000
Volume of QIAGEN Protease (µL)	1	3	5	10	30	50	60

2.4.2 DNA extracted from NBS cards or Guthrie cards

Zymo ZR DNA-card Extraction Kit (Zymo Research Corp, USA) was used to extract DNA from NBS cards. The manufacturer's protocol was modified and adapted to improve DNA yield from our samples. Prior to the day of extraction, eight 3 mm newborn screening card blood spots were placed in a 2 mL Eppendorf tube containing Bashing-Bead™ and incubated in 360 μL of phosphate buffered saline (NaCl, Na_2HPO_4, NaH_2PO_4, RO H_2O) and 40 µL of proteinase K (20 mg/mL) at 37°C overnight. The next day, 400 µL of Zymo ZR lysis solution was added to the tube and the spots were homogenised using QIAGEN® TissueLyser II (QIAGEN®, Germany) for 20 seconds at a frequency of 30 oscillations/second. The tube was centrifuged for 1 minute, followed by addition of 390 μL of proprietary digestion buffer and 10 µL of proteinase K (20 mg/mL). The tube was again mixed and then incubated at 55°C for 30 minutes. After incubation, the tube was left to cool at room temperature for 3-4 minutes before being centrifuged for 1 minute. A portion of the supernatant (~650 μL) was transferred to a 5 mL falcon tube containing 1.3 mL Zymo ZR DNA isolation buffer. A portion of this mixture (~650 μL) was transferred to a Zymo-Spin IC column in a collection tube and centrifuged for 1 minute at 18,400 *g*. Flow through was discarded and this step was repeated until the entire volume of the mixture in the 5 mL falcon tube had passed through the column. Once all the flow through had passed through the column, 200 μL of Zymo ZR DNA Wash Buffer was added to the spin column, centrifuged for 1 minute at 18,400 *g* and repeated. The spin column was transferred to a new 1.5 mL microcentrifuge tube and allowed to dry before 20 μL of pre-warmed (55- 60°C) Zymo ZR DNA elution buffer was added into the column. The tube was incubated at room temperature for 15 minutes, followed by centrifugation for 2 minutes at 18, 400 *g*. Another 20 μL of pre-warmed Zymo ZR DNA

elution buffer was added into the spin column and left at room temperature for 15 minutes. The elution containing DNA was then centrifuged for 2 minute at 18, 400 *g* before long term storage at -80°C.

2.4.3 DNA quantification

Absorbance spectrophotometry using NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific, USA) was used to determine quality and yield of extracted DNA. NanoDrop™ is an ultraviolet absorbance-based quantification system and is used primarily to check for the purity of extracted DNA. Nucleic acid quantitation of 2 µL of DNA extract was carried out using the NanoDrop™, including the ND-1000 version 3.8.1 software package. The sample absorbance at a wavelength of 260 nm was a measure DNA yield. As a measure of DNA purity, the ratios of sample absorbance at 260/280 nm and 260/230 nm were taken into consideration. Extracted DNA with a 260/280 ratio of 1.7 to 1.9 was accepted as sufficiently pure and free from protein contamination. The 260/230 ratio serves as a secondary measure of purity and a value of ~ 2.0 is taken to be acceptable.

2.5 Genotyping

Using the extracted DNA, SNP genotyping was carried out using a commercially available multiplex genotyping technology, Agena Bioscience MassARRAY® Genotyping platform (Hamburg, Germany). This platform uses single base extension reactions using the iPLEX® chemistry along with matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry to identify genotypes.

In brief, the iPLEX® genotyping chemistry assay comprised of three main steps: a polymerase chain reaction (PCR), iPLEX® extension reaction and MALDI-TOF mass spectrometry analysis, each detailed further in the following sections. The MALDI-TOF mass spectrometry system determines the mass of the detected extended base and translates the mass of the observed primers into the allele present at the polymorphic site of interest using the software SpectroTYPER®. The pipeline of the genotyping chemistry is illustrated in Figure 2.4. Consumables used for these processes are part of the iPLEX® Gold Genotyping Reagent Set (Agena Bioscience, Germany).

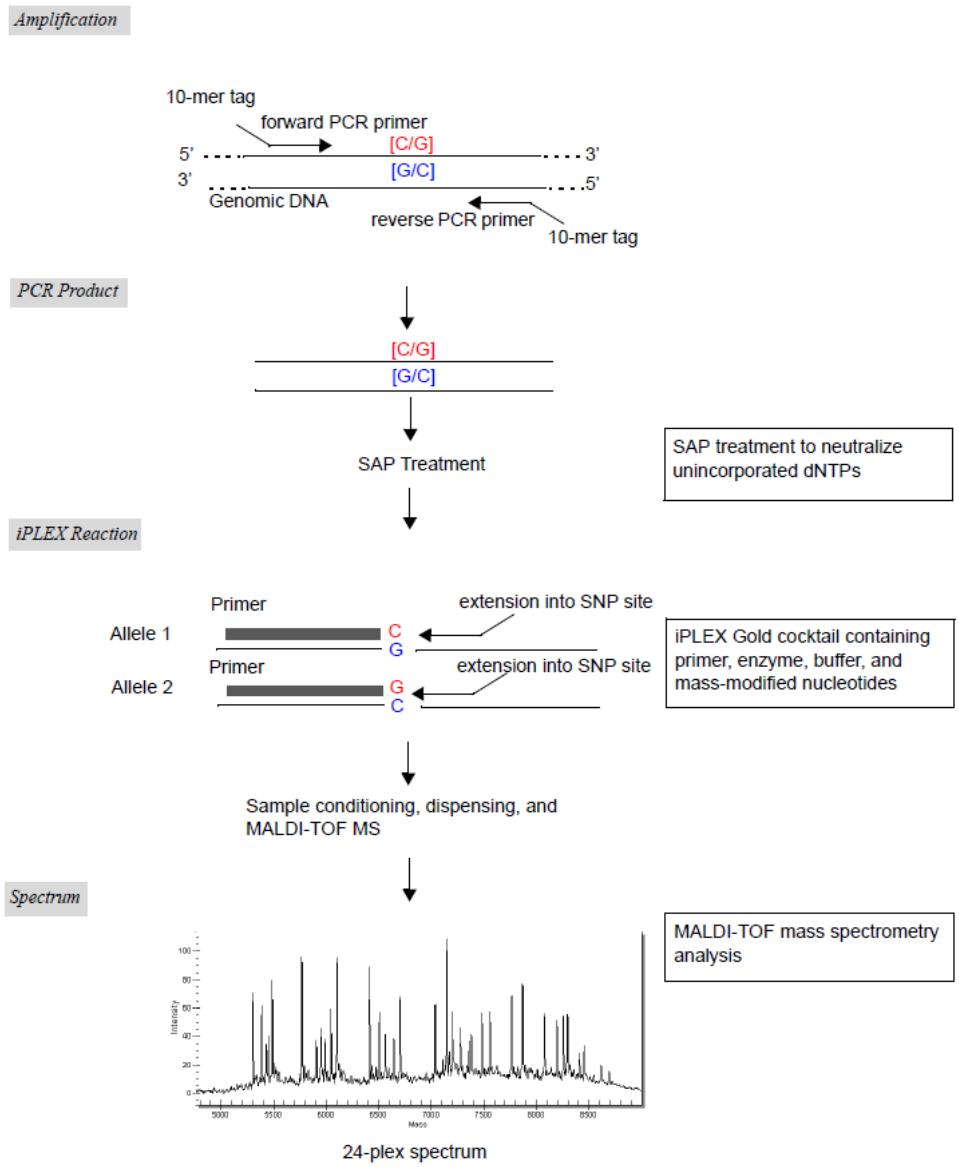

Figure 2.4 Summary of the iPLEX® gold reaction processes (162).

2.5.1 DNA amplification

Firstly, genetic loci of interests were amplified from genomic DNA using multiplex PCR. A PCR master mix was made up on ice according to measurements detailed in Table 2.2. To each well of a 384-well microtiter plate, 4 μL of PCR master mix was added to 1 μL of the appropriate genomic DNA sample (10-20 ng/μL) on ice. The plate was centrifuged at 200 *g* for 1 minute before placing into a GeneAmp® PCR system 9700 thermocycler (Applied Biosystems, USA) for PCR. Thermocycler conditions used for DNA amplification are as follows:

94 °C for 15 minutes
45 cycles of (94 °C 20 s, 56 °C 30 s, 72 °C 1 minutes)
72 °C for 3 minutes
4 °C hold

Once completed, the plate was kept at -20˚C until ready to proceed to the next step.

Table 2.2 Concentrations of reagents in iPLEX® PCR Master Mix

Reagent	**Concentration in 5 μL**	**Volume (1 reaction)**	**Volume (384 reactions)[a]**
Water (HPLC grade)	NA	1.9 μL	875.5 μL
10x PCR Buffer with 20 mM $MgCl_2$	1 x (2 mM $MgCl_2$)	0.5 μL	230.4 μL
$MgCl_2$ (25 mM)[b]	2 mM	0.4 μL	184.3 μL
dNTP mix (25 mM each)[c]	500 μM	0.1 μL	46.1 μL
Primer mix (500 nM each)	100 nM	1.0 μL	460.8 μL
PCR enzyme (5 U/ μL)[d]	0.5 U/reaction	0.1 μL	46.1 μL
Total Volume		4.0 μL	1843.2 μL

[a] Volumes include a 38% overhang to account for pipetting losses

[b] The final $MgCl_2$ concentration is 4.0 mM, 2.0 mM from the PCR buffer and 2.0 mM from the $MgCl_2$

[c] No more than 5 freeze-thaw cycles

[d] For plexes more than 27, increase MassARRAY® PCR enzyme to 1U/reaction = 0.2 μL

2.5.2 Shrimp alkaline phosphatase purification

Following PCR, shrimp alkaline phosphatase (SAP) was used to dephosphorylate excess nucleotides that were not incorporated in the PCR reaction. In doing so, this prevents additional nucleotides from attaching to the unincorporated nucleotides in the iPLEX® extension reaction. Therefore, ensuring that only terminator nucleotides are available for extension.

SAP enzyme solution was prepared on ice according to measurements reagents listed in Table 2.3. To each well of the PCR 384-well sample plates, 2 *u*L of the SAP enzyme solution was added. The plate was again centrifuged at 200 *g* for 1 minute before loading onto the thermocycler to be incubated at the following conditions: 37º C for 40 minutes, 85º C for 5 minutes, 4º C on hold.

Table 2.3 Concentrations of reagents in iPLEX® SAP Master Mix

Reagent	Volume (1 reaction)	Volume (384 reactions) [b]
Water (HPLC grade)	1.53 μL	810.9 μL
SAP Buffer (10x)	0.17 μL	90.1 μL
SAP enzyme (1.7 U/ μL)	0.30 μL	159.0 μL
Total Volume	2.00 μL	1060.0 μL

[a] Same amounts for both low and high plex iPLEX® reactions

[b] Volumes include a 38% overhang to account for pipetting losses

2.5.3 iPLEX® single base extension reaction

The primer extension (iPLEX®) reaction involved addition of mass-modified nucleotides to the amplified DNA. The extension primers were annealed directly adjacent to each polymorphic site of interest and were extended and terminated by a single complementary base. The terminator nucleotide prevents any further nucleotides from further extending the DNA fragment. These terminator bases were ‘mass-modified’ to enable mass spectrometry to identify mass differences between fragments differing by a single base (163).

2.5.3.1 iPLEX® Gold Reaction master mix

The quantity required for iPLEX® Gold reaction master mix differs by the plex level as shown in Table 2.4. Once the appropriate level of master mix had been made, 2 *u*L of master mix was added to each well of the 384-well plate containing the PCR and SAP products. The plate was centrifuged at 200 *g* for 1 minute and then thermocycled at the following conditions:

94 °C for 30 s
40 cycles of (94 °C 5 s, (5 cycles of 52 °C 5 s, 80 °C 5 s))
72 °C for 3 minutes
4 °C hold

Table 2.4 Concentrations of reagents in iPLEX® Gold reaction primer extension reaction master mix for low [lex (1-18 plex) and high plex (19-36+) volumes

Reagent	**Low plex Volume (1 reaction)**	**High plex Volume (1 reaction)**
Water (HPLC grade)	0.7395 μL	0.619 μL
iPLEX Buffer Plus (10x)	0.200 μL	0.200 μL
iPLEX Termination mix	0.100 μL	0.200 μL
UEP mix (7μM: 14μM)	0.94 μL	0.94 μL
iPLEX enzyme	0.0205 μL	0.041 μL
Total Volume	2.000 μL	2.000 μL

[a] Volumes include a 38% overhang to account for pipetting losses

2.5.3.2 Purification of iPLEX® Gold reaction products

The last step in the genotyping process involved adding clean resin to the plate. The addition of resin removes any excess salt adducts such as Na^+ and K^+ that may cause high background noise in the mass spectra.

The sample microtiter plate was centrifuged at 2000 *g* for 1 minute. Thereafter, 16 *u*L of water was added to each well of the microtiter plate. Meanwhile, clean resin was spread

into the wells of a 384-well 'dimple plate' and left to dry for at least 20 minutes. Once dry, the resin was transferred from the dimple plate to the sample plate. The sample plate was then rotated for at least 5 minutes at room temperature and centrifuged at 3200 *g* for 5 minutes just before transferring of reaction products to a SpectroCHIP® (Agena Bioscience, Germany) for measurement using the MassARRAY® Nanodispenser RS1000 (Agena Bioscience, Germany).

2.5.4 Nanodispensing of iPLEX® Gold reaction products

Nanodispensing of the iPLEX® Gold reaction products onto a SpectroCHIP® (Agena Bioscience, Germany) was carried out using MassARRAY® Nanodispenser (Agena Bioscience, Germany). In short, a small volume (~25 nL) of sample was spotted onto the chip. The chip was placed into a mass spectrometer to determine the mass of the DNA fragments through the use of the Sequenom MassARRAY® MALDI-TOF system run by Dr Benjamin Ong, MCRI Agena Bioscience facility manager (Melbourne, Australia). Each spot on the chip was fired at with UV laser light in short pulses under vacuum. The relative time of flight for each sample analyte to travel from the bottom of the vacuum tube to the top corresponds to the mass of the extended primer, allowing the nucleotide base present at the polymorphic site of interest to be determined (163). The Agena Bioscience MassARRAY® Typer automatically translates the mass of the observed primers into a genotype for each sample.

2.6 Statistical analyses

This section outlines the software and common statistical methods used throughout this thesis. Detailed methods and statistical analysis approaches relevant to individual chapters are presented within each chapter (Chapters 3 to 7).

2.6.1 Stata

Stata is a multi-purpose data analysis and statistical software (StataCorp, College Station, Texas, USA). Stata was used to manage data obtained from questionnaires and statistical analysis of environmental exposures.

Chi-square analysis and binary logistic regression were done to obtain odds ratios with 95% confidence intervals. Multiple logistic regressions were used to adjust for any possible confounding factors. Potential confounding factors were only included in the analyses if they resulted in a 10% change in the odds ratio for the association of interest.

Any potential effect modifiers between exposure variables and outcome were assessed using interaction terms. Likelihood ratio tests were then used to compare models with and without interaction terms. A p-value <0.05 was used to determine if the final model should be stratified by the effect modifier.

Stata I\C 14 and 15 were used for all statistical analyses.

2.6.2 PLINK

PLINK (V1.90) is a publicly available whole genome association analysis tool (164). Genotyping data obtained was input into PLINK in a binary format to obtain genotypic and allelic frequencies. Hardy-Weinberg equilibrium tests, sample and SNP genotyping success were computed as quality control based on pre-defined criteria. Samples and SNPs not meeting the criteria were excluded from analyses. Summary statistics and association analysis between food allergy and individual SNPs were also carried out. PLINK was also used to obtain Bonferroni corrected p-value, adjusted for multiple testing.

The main association analysis carried out using PLINK compared allele frequencies between cases and controls by means of the basic allelic test. For SNPs that showed some evidence of association (nominal p-value < 0.1), association tests based on additive (Cochrane-Armitage test for trend), dominant and recessive models were also carried out to investigate whether the risk of food allergy conferred by the particular SNP might act via a different trait model. Detailed information on the genetic association analyses are described in Chapter 7.

Genotyped data was also imported into Stata I\C 15 for additional analyses. Significance was defined at p-value < 0.05.

Chapter 3 Characterising prevalence of allergic diseases in Asian and Caucasian children in Australia

This chapter is presented in the form of a manuscript that was accepted for publication in the Journal of Allergy and Clinical Immunology: In Practice in July 2018. This body of research was also presented in an Oral session at the European Academy of Allergy and Clinical Immunology 2017, Paediatric Asthma and Allergy Meeting (EAACI-PAAM) meeting, held in London, UK in October 2017. The presentation was also awarded the Abstract award.

3.1 Study rationale

The HealthNuts study has shown that, in Australia, infants with Asian born parents are three times more likely to develop food allergy than infants with Caucasian-born parents. Food allergy and eczema often manifests concurrently and may be a potential risk factor for other allergic comorbidities such as hay fever and asthma which happen later in life, as shown by the atopic march. However, to date, no studies have looked at whether the increased risk in food allergy in infancy in the Asian population translates to an increased risk of childhood allergic comorbidities later in life. This chapter characterises the childhood allergic comorbidities including aeroallergen sensitisation at age 6 years.

3.2 Research questions

This chapter will answer the following research questions:

1. What is the prevalence of asthma, allergic rhinitis, and aeroallergen sensitisation at age 6 in the infants with Asian-born parents living in Australia compared to those with Caucasian-born parents living in Australia?
2. Is infant food allergy and eczema status at age 1 year associated with childhood asthma and allergic rhinitis at age 6 years in the infants with Asian-born parents living in Australia?
3. How do these associations compare to infants with Caucasian-born parents living in Australia?

3.3 Manuscript: Children with East Asian-born parents have an increased risk of allergy but may not have more asthma in early childhood

Noor H. A. Suaini, BSc (Hons) [1,2], Jennifer J. Koplin, PhD[2,3], Rachel L. Peters, PhD[1,2], Mari Sasaki, MD[2], Justine A. Ellis, PhD[1,4,5], David J. Martino, PhD[1,2], Adrian J. Lowe, PhD[3], Mimi L. K. Tang, MBBS, FRACP, FRCPA, PhD[1,2,6], Anne-Louise Ponsonby, BMedSci, MBBS, PhD[1,3], Lyle C. Gurrin, PhD[3], Shyamali C. Dharmage, MBBS, MSc, MD, PhD[3], Katrina J. Allen, MBBS, FRACP, PhD, FAAAAI, FAHMS[1,2,6,7], for the HealthNuts study group

Affiliations:

[1]Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia

[2]Centre for Food and Allergy Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[3]The School of Population and Global Health, University of Melbourne, Carlton

[4]Centre for Social and Early Emotional Development, Faculty of Health, Deakin University, Burwood, Victoria, Australia.

[5]Genes, Environment & Complex Disease, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[6]Department of Allergy and Clinical Immunology, Royal Children's Hospital, Parkville, Victoria, Australia

[7]University of Manchester, Manchester, United Kingdom.

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Disclosure of potential conflict of interest:

M. L. K. Tang is on the Nestle Nutrition Institute Medical Advisory Board Oceania; is a past member of the Danone Nutricia Global Scientific Advisory Board; has received consultancy fees from Deerfield Consulting, GLG Consulting, and Bayer; is employed by and has stock/stock options in ProTA Therapeutics; has received lecture fees from Danone Nutricia and Nestle Health Sciences; has a patent owned by Murdoch Children's Research Institute; received royalties from Wilkinson Publishing; and has received payment for developing educational presentations from MD Linx. K. J. Allen serves as a consultant for Nestle, ThermoFisher, AspenCare, Before Brands, and Nutricia. The rest of the authors declare that they have no relevant conflicts of interest.

Keywords:

allergic rhinitis; ancestry; Asians; asthma; children; eczema; ethnicity; food allergy; hay fever

Abbreviations:

ISAAC: International Study of Asthma and Allergies in Childhood

SACC: Standard Australian Classification of Countries

SPT: skin prick test

OFC: oral food challenge

PR: prevalence ratios

Acknowledgements:

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Highlights box

1. **What is already known about this topic?**

Infants with East Asian-born parents living in Melbourne have a higher risk of IgE-mediated food allergy than those with Caucasian-born parents. It is unclear if this increased risk extends to other allergic diseases later in childhood.

2. **What does this article add to our knowledge?**

IgE-mediated food allergy and eczema at age 1 increase the risk of asthma and allergic rhinitis at age 6, but this association was not modified by ancestry. Children with East Asian-born parents do go on to have more allergic diseases at age 6 years, while atopic asthma appears to be similar compared to children with Caucasian-born parents.

3. **How does this study impact current management guidelines**

Clinicians will be able to use this data to better inform parents of East Asian children on the progression of their child's allergic diseases early in their childhood. Future prevention and management approaches to allergic diseases can be tailor-made and targeted to this population.

3.3.1 Abstract

Background: We previously reported that infants with Asian-born parents are three times more likely to have IgE-mediated food allergy than those with Australian-born parents. It is unknown whether this translates to increased risk of other allergic diseases later in childhood and whether ancestry interacts with other risk factors for allergic disease development.

Objective: To compare prevalence and risk factors for allergic rhinitis, asthma and aeroallergen sensitisation at age 6 between children with East Asian-born and Caucasian-born parents.

Methods: 5276 1-year-old infants were recruited into a population-based longitudinal study of allergy. 4455 children participated in age 6 follow-up (84.4%), including 3015 with Caucasian-born parents and 415 with East Asian-born parents. Children underwent skin prick tests to aeroallergens and questionnaires captured data on asthma, eczema and allergic rhinitis.

Results: Compared to children with Caucasian-born parents, children of East Asian-born parents had more allergic rhinitis (19.9% [95%CI 14.9-26] versus 9.3% [95%CI 8-10.8], p<0.001) and aeroallergen sensitisation (64.3% [95%CI 57.5-70.5] versus 34.4% [95%CI 32.2-36.7], p<0.001) at age 6. Asthma was similar in both groups (9.1% [95%CI 6.2-13.2] versus 11.7% [95%CI 10.4-13.1]), p=0.21. Children with IgE-mediated food allergy and eczema in infancy were 3 times more likely to have asthma and 2 times more likely to have allergic rhinitis at age 6, irrespective of ancestry.

Conclusions: Children of East Asian ancestry born in Australia have a higher burden of most allergic diseases in the first 6 years of life, while asthma may follow a different pattern. IgE-mediated food allergy and eczema at age 1 increase the risk of asthma and allergic rhinitis irrespective of ancestry.

3.3.2 Introduction

There is increasing evidence that risk of allergic disease differs according to both ancestry and the environment in early life. We previously showed that children of Asian descent who were born in Australia had very high rates of eczema and food allergy in early life (166, 167), with up to 50% of infants with both parents born in East Asia having eczema and 25% having challenge-confirmed food allergy by 1 year of age (49). In contrast, children who were born in Asia and subsequently migrated to Australia in early childhood appeared to be protected from developing food allergy (166). This difference in allergy prevalence by both ancestry and country of birth is not limited to Australia. A study in the United States (US) found that children born outside the US had a lower risk of food sensitisation than those born in the US, while the highest risk of food sensitisation was seen among children born in the US to migrant parents (21). Another study in the United Kingdom (UK), found an over-representation of non-Caucasian children in a paediatric allergy clinic (22).

Despite the amplified burden of allergic disease in infancy among children of Asian ancestry who are born in Australia, little is known about their risk of allergic disease later in life. In general, it has been estimated that of those with eczema during the first 4 years of life, around one-third progress to develop asthma and two-thirds develop allergic rhinitis (168-170). It is also generally accepted that early life food allergy and eczema clinically co-associate (171). Less is known about the role of food allergy in the development of the atopic march to asthma and allergic rhinitis, although we have previously reported that children with food allergy at age 1 year were more likely to have a doctor-diagnosis of asthma at 4 years of age and that the risk was highest for children with food allergy and co-existent eczema in infancy (172). It is not known whether the atopic march is modified by ancestry or country of birth.

As such, the aims of this study were: (1) to compare the prevalence of allergic rhinitis, asthma and aeroallergen sensitisation in Australian children with East Asian-born and Caucasian-born parents at age 6 years and (2) to investigate whether the associations between IgE-mediated food allergy and eczema in the first year of life and allergic disease at age 6 years differ depending on ancestry, using data from our recently completed age 6 follow-up of the HealthNuts population-based longitudinal study of allergic disease.

3.3.3 Methods

Study population

The HealthNuts study is a longitudinal population-based cohort study of allergic disease in Melbourne, Australia. The recruitment process has previously been described in detail (156). Briefly, 5,276 12-month-old infants were recruited from immunisation clinics around Melbourne (74% participation rate). All infants underwent a skin prick test (SPT) at recruitment to four foods (egg, peanut, sesame, shrimp/cow's milk) (ALK-Abello, Madrid, Spain). Infants with a detectable wheal size ≥ 1 mm to any of the foods were invited to the HealthNuts clinic at the Royal Children's Hospital, Melbourne for an oral food challenge (OFC) to ascertain their food allergy status. OFCs were carried out using a predetermined protocol as previously described (156, 157).

Age 6 year *follow-up*

At 6 years of age all children (N=5276) were invited to attend a HealthNuts allergy clinic at the Royal Children's Hospital. Home visit assessments were offered for those who were unable to attend the hospital. Parents completed a questionnaire which included general information about the child as well as family and child's history of asthma, wheeze, allergic rhinitis, and eczema. We also incorporated questions on asthma, eczema and allergic rhinitis from the validated International Study of Asthma and Allergies in Childhood (ISAAC) (160). Those who did not complete the full questionnaire were given the option to complete a short telephone questionnaire, which asked a limited number of questions including whether the child had ever been diagnosed with asthma or allergic rhinitis.

Skin prick tests

At age 6 years, all children who participated in an assessment, either at the Royal Children's Hospital or home visit, underwent a skin prick test to peanut, egg, sesame, soy, almond, cashew, hazelnut, shellfish, cow's milk, wheat, house dust mite, rye grass, bermuda grass, cat hair, alternaria, birch mix, cladosporium and dog hair. Aeroallergen sensitisations were determined in the whole cohort only at age 6. SPTs were carried out with

a single-tine lancet (Stallergenes, Antony, France) on the child's back using allergen extracts (ALK-Abello, Madrid, Spain) including a positive control (10 mg/mL histamine) and a 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 and then subtracting the negative control SPT diameter.

Definitions

Ancestry: Parental country of birth was used as a proxy for ancestry background. We have shown previously that parental country of birth information correlated well with genetically inferred ancestry (93.7% correlation for "Caucasians" and 93.0% correlation for "Asians") (130). For analysis, we focused on two groups, East Asians and Caucasians. Caucasians refer to children with both parents being born in Australia, UK or Europe. The East Asian group was made up of children with one or two East Asian-born parents, as defined in one of our previous study (49). Our East Asian definition included countries in the North East Asia region such as China, Hong Kong, Japan, Taiwan, Macau, North Korea, South Korea and South East Asia region of Vietnam, Philippines, Singapore, Thailand, Indonesia, Malaysia, Cambodia and Laos. Groupings were based on the Standard Australian Classification of Countries (SACC) which were developed to be relevant to Australia's multicultural society for use in analysing Australian-based country of origin data (161). Groups in the SACC comprise geographically proximate countries which have broadly similar social, cultural, economic and political characteristics. All other country groups (e.g. South Asians, Middle East and Africa) were not included in the analyses due to small numbers. We also correlated parental country of birth with self-reported grandparents' ancestry. 87% of children classified in the East Asian group had at least one set of grandparents reporting Asian as their ancestry whereas 80% of those in the Caucasian group had both sets of grandparents reporting as Caucasian.

Definitions for allergy at age 1 year

Food allergy: Defined as a positive OFC outcome or recent reaction consistent with our OFC stopping criteria (157) to peanut, sesame or egg, in conjunction with a positive sensitisation test (SPT with wheal size $\geq$ 2 mm greater than negative control and/or sIgE > 0.35 kU/L). A positive OFC was defined as: more than 3 non-contact urticarial reactions

lasting more than 5 minutes, angioedema, vomiting, or anaphylaxis, within 2 hours of the last challenge dose. Upon discharge, those with a negative challenge (able to tolerate top dose of challenged food without any subsequent allergic reactions) were administered a single serving of the challenged food at home for 7 days to capture any late reactions.

Infantile eczema: Defined as parent report doctor diagnosis of eczema during the first year of life.

Definitions for allergy at age 6

Eczema: Parent report of an itchy rash in the last 12 months that affected typical eczema locations, such as folds of elbows and knees (158).

Aeroallergen sensitisation: Positive skin prick test wheal ≥ 3mm greater than negative control for any of the following aeroallergens: house dust mite, rye grass, bermuda grass, cat hair, alternaria, birch mix, cladosporium, dog hair.

Allergic rhinitis: was defined as nose symptoms in the last 12 months accompanied by itchy watery eyes (158, 160) in the presence of aeroallergen sensitisation.

Asthma: was defined as parent report of a doctor diagnosis of asthma AND either wheeze or use of asthma medication in the last 12 months.

Atopic asthma: We also further classified asthma into atopic asthma (asthma with aeroallergen sensitisation) and non-atopic asthma (asthma with no aeroallergen sensitisation) (173, 174).

Statistical Analysis

This is a post-hoc cohort analysis, as a follow-up to our previous study that found children with East Asian-born parents have an increased risk of food allergy at age 1 year (49).

Prevalence estimates

The prevalence of allergy outcomes at age 6 years in each ancestry group was estimated as the observed proportion with 95% confidence intervals generated using the normal

approximation to the binomial distribution. To control for the potential impact of differential loss to follow-up, we adjusted for differences in demographic characteristics and other potential risk factors between participants who completed the full questionnaire at age 6 years and those who were lost to follow-up/not included in the analysis, using the inverse probability weighting method described by Little and Rubin (175) (see online repository for details).

Regression models

For each ancestry, the association between allergy status at age 1 (food allergy and infantile eczema) and risk of asthma and allergic rhinitis at age 6 was estimated using binomial regressions (a generalised linear model with a logarithm link function) to obtain estimates of prevalence ratios (PR) and 95% confidence intervals for the corresponding population parameter. Interaction analyses between ancestry and food allergy and eczema at age 1 year were tested by adding product terms to the regression model.

Wheezing in the first year of life could be an early indicator of asthma (176). Therefore, to investigate the longitudinal association between food allergy at age 1 year and the subsequent development of asthma, i.e. to assess newly incident disease after age 1, we repeated our analyses after excluding children with wheezing in the first year of life.

The regression analyses were adjusted for the following potential confounders based on previous published literature: sex, socioeconomic status and parent's or sibling's history of asthma, eczema, allergic rhinitis or food allergy. Additional potential confounders considered include presence of cat or dog in the household, household smoking, maternal smoking during pregnancy, mode of delivery, antibiotic use in the first year of life, birthweight (< 2500 grams vs ≥ 2500 grams), duration of breastfeeding, number of siblings and season of birth (winter vs other) (177, 178). These were included in the regression models if they changed the magnitude of the association between the exposure and outcome by more than 10% on the prevalence ratio scale.

Sensitivity analyses

We performed the following sensitivity analyses for our regression models, to examine the potential impact of loss to follow-up on our results, and to examine whether our findings were robust to different definitions of asthma and allergic rhinitis:

1) To control for the potential impact of differential loss to follow-up, we adjusted for differences in demographic characteristics and other potential risk factors between participants who completed the full questionnaire at age 6 years and those who were lost to follow-up/not included in the analysis, using the inverse probability weighting method described by Little and Rubin (175) (see online repository for details).

2) To reduce the amount of missing data at age 6 years, we combined data on asthma and allergic rhinitis diagnosis from the full and short questionnaire, and repeated the analysis to examine consistency of our results while using data from a greater percentage of the original cohort.

3) To examine whether our findings were robust when using different definitions of asthma and allergic rhinitis, we repeated the analyses using the ISAAC definitions of asthma (answering Yes to both questions "*Has your child ever had wheezing or whistling in the chest at any time in the past?*" and "*Has your child had wheezing in the past 12 months?*") and allergic rhinitis (answering Yes to both questions "*Has your child ever had sneezing or runny or blocked nose when he/she did not have a cold or flu?*" and "*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/flu?") (160).*

The results of each sensitivity analysis are reported in the online repository.

All analyses were performed using Stata 15 for Windows (StataCorp LP, College Station, TX, USA). Venn diagrams were obtained using Venn Diagram Plotter (PNNL, Richland, WA, USA) (179). The Venn Diagram Plotter is supported by the W.R. Wiley Environmental Molecular Science Laboratory, a national scientific user facility sponsored by the U.S. Department of Energy's Office of Biological and Environmental Research and located at PNNL. PNNL is operated by Battelle Memorial Institute for the U.S. Department of Energy under contract DE-AC05-76RL0 1830.

Ethics Approval

Ethics approval was obtained from the Royal Children's Hospital Human Research Ethics Committee (reference no. 27047 and 32294).

3.3.4 Results

Study population

Of the 5276 infants recruited at 12-months, 84% participated in the age 6 years follow-up, with a majority of these answering the full questionnaire (n=3663). An additional 605 participants completed the short questionnaires and were included in our sensitivity analyses (see Figure E1 in the Online Repository). Assessments including SPT were completed by 3233 children at age 6 years. Demographic and baseline characteristics of the study population are provided in Table 3.1.

Table 3.1 Demographics and baseline characteristics of study population included in main analyses which included children with full questionnaire only

	Caucasian (n=2620)	**East Asian (n=352)**	**P value**
Demographics			
Birthweight, *grams* (mean, range)	3451 (630-5160)	3208 (692-4690)	0.032
Male	1323 (50.7)	204 (58.6)	0.005
Infant's season of birth			
Summer	597 (22.8)	77 (21.9)	0.319
Autumn	638 (24.4)	75 (21.3)	
Winter	701 (26.8)	93 (26.4)	
Spring	683 (26.1)	107 (30.4)	
Caesarean delivery	863 (33)	110 (31.3)	0.53
≤ 36 weeks gestation	144 (5.7)	23 (7)	0.354
Quintiles of SEIFA disadvantage			
1 (most disadvantaged)	451 (17.2)	53 (15.1)	0.416
2	538 (20.6)	84 (23.9)	
3	577 (22.1)	79 (22.4)	
4	548 (21)	64 (18.2)	

	Caucasian (n=2620)	**East Asian (n=352)**	**P value**
5 (least disadvantaged)	501 (19.2)	72 (20.5)	
Family history of asthma, eczema, allergic rhinitis or food allergy	1956 (74.7)	244 (69.3)	0.032
Environmental Exposure			
Number of siblings			
No siblings	1306 (50.3)	189 (54)	0.213
1 sibling	864 (33.2)	116 (33.1)	
2 siblings	337 (13)	32 (9.1)	
3 or more siblings	92 (3.5)	13 (3.7)	
Use of antibiotics	1293 (51)	150 (44.5)	0.026
Childcare attendance	741 (28.3)	78 (22.2)	0.016
Cat ownership	514 (19.6)	21 (6)	<0.001
Dog ownership	976 (37.3)	52 (14.8)	<0.001
Household smoking	504 (19.3)	65 (18.5)	0.703
Maternal smoking during pregnancy	105 (4)	2 (0.6)	0.001
Infant's diet			
Any breastfeeding	2498 (95.8)	341 (97.2)	0.233
Exclusive breastfeeding	1398 (61.3)	148 (47.9)	<0.001
Any formula feeding	1856 (76.3)	285 (84.6)	0.001

Prevalence of allergic diseases at age 6

Allergic rhinitis and eczema at 6 years of age were more common in East Asian children than Caucasian children (Figure 3.1). Allergic rhinitis was present in 19.9% (95% CI 14.9-26.0) of East Asian children and 9.3% (95% CI 8.0-10.8) of Caucasian children ($p<0.001$). Eczema was present in 26.0% (95% CI 21.0-31.7) of East Asian children and 12.8% (95% CI 11.5-14.3) of Caucasian children ($p<0.001$).

The prevalence of aeroallergen sensitisation was also higher in East Asian children, with 64.3% (95% CI 57.5-70.5) sensitised to at least one aeroallergen compared to 34.4% (95% CI 32.2-36.7) of Caucasian children ($p<0.001$). When aeroallergens were examined individually, sensitisation to each of the tested allergens was higher in the Asian children (data not shown).

Conversely, asthma prevalence was similar in East Asian and Caucasian children, affecting 9.1% (95% CI 6.2-13.2) of East Asian children and 11.7% (95% CI 10.4-13.1) of Caucasian children ($p=0.21$; Figure 3.1). When assessed as asthma phenotypes, East Asian children had a lower prevalence of non-atopic asthma (1.9%, 95% CI 0.7-5.1) compared with Caucasian children (4.5%, 95% CI 3.6-5.7), ($p=0.096$) while the prevalence of atopic asthma was similar in both groups (8.9% versus 7.9%, $p=0.607$).

An increase in prevalence of allergic disease was observed when comparing children with one versus two East Asian-born parent (Online Repository Figure E2).

Further, among East Asian infants with eczema, food allergy or both at age 1, 12.8% (95% CI 8.1-19.6) had asthma at age 6, compared to 17.8% (95%CI 15.0-20.9) of Caucasian infants with eczema, food allergy or both at age 1 ($p=0.165$).

Relationship between aeroallergen sensitisation and asthma

The overlap between aeroallergen sensitisation and asthma in Caucasian and East Asian children is shown in Figure 3.2. Overall, 70% of East Asian children had either aeroallergen sensitisation and/or asthma compared with only 41% of Caucasian children. This was due to a high prevalence of aeroallergen sensitisation in the East Asian group. However, fewer of the aeroallergen-sensitised East Asian children also had asthma compared with the aeroallergen-sensitised Caucasian children (15% versus 24%, p=0.01).

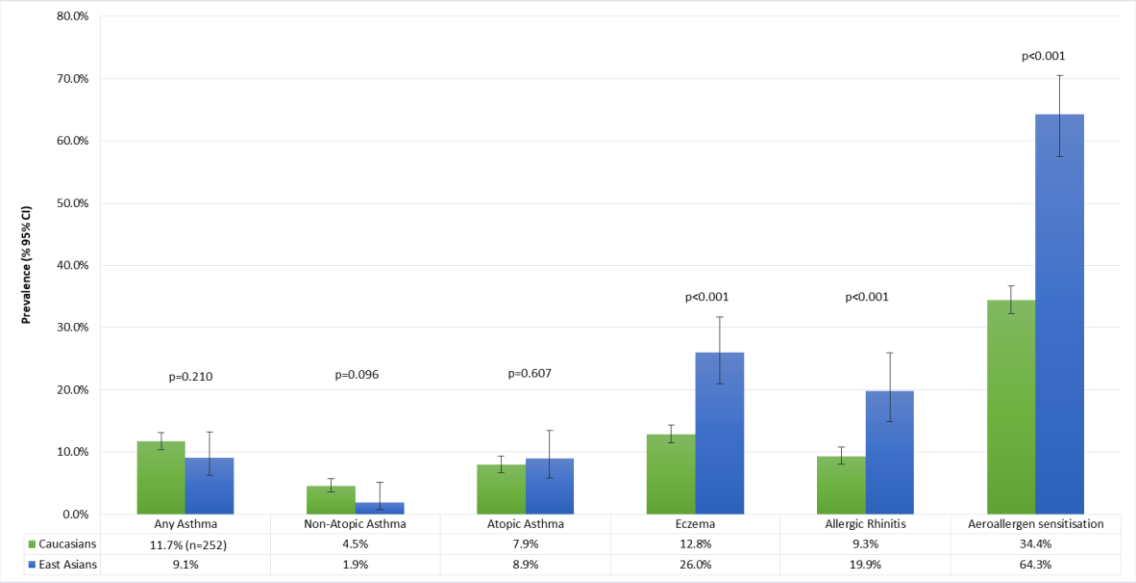

Figure 3.1 Weighted prevalence of asthma, allergic rhinitis, eczema and atopic asthma at age 6, stratified by ancestry, with 95% CI. Prevalence estimates were adjusted (weighted) for differences in demographic characteristics and other potential risk factors between participants who completed the full questionnaire at age 6 years and those who were lost to follow-up/not included in the analysis, using the inverse probability weighting method described by Little and Rubin (175) (see online repository for details).

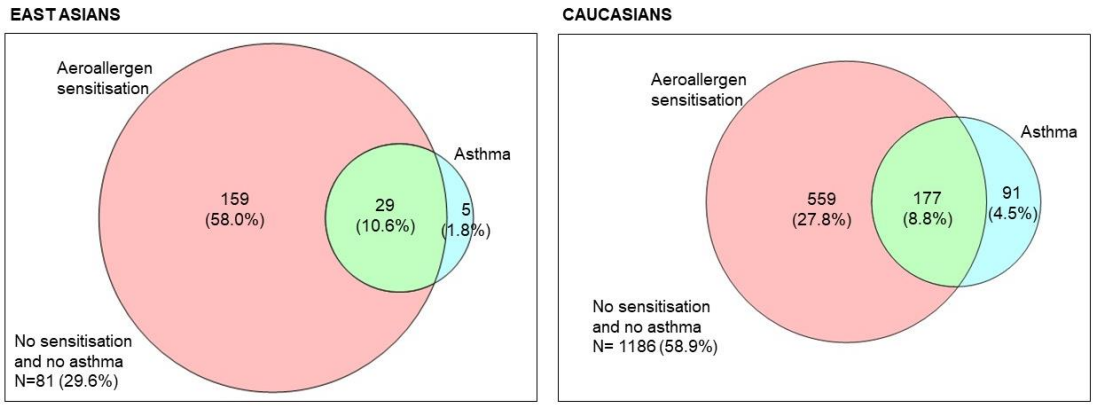

Figure 3.2 Aeroallergen sensitisation and asthma at age 6 years in children with East Asian-born parents (n=274) and Caucasian-born parents (n=2013).

Associations between early life eczema and food allergy status and asthma at age 6

Table 3.2 shows the relationship between infantile food allergy and eczema and diagnosed asthma at age 6 years. Caucasian children with both eczema and food allergy at age 1 were three times as likely to have asthma at age 6 compared to those with no eczema and no food allergy at age 1 (PR 3.56, 95% CI 2.72-4.67, p<0.001). The magnitude of association was similar in East Asian children (PR 3.12, 95% CI 1.21-8.08, p=0.019). There was no evidence that the association between food allergy and eczema status at age 1 and asthma at age 6 differed by ancestry. The associations observed in each ancestry group were broadly similar after excluding children with a history of wheeze in the first year of life, although the association seen in children with eczema alone was reduced (Table 3.2).

Caucasian children with food allergy or eczema alone were twice as likely to have asthma at age 6. Food allergy alone remain associated with asthma after excluding Caucasian children with history of wheeze (Table 3.2). These findings were not observed in East Asian children.

Similar results were observed in a sensitivity analysis which included sampling weights to adjust for differences in those lost to follow-up compared to those who participated at age 6 years (see Table E1 in the Online Repository), as well as in sensitivity analyses using broader definitions of asthma (see Table E2 in the Online Repository) and ISAAC definitions (see Table E3 in the Online Repository).

Table 3.2 Association between food allergy and eczema at 1 year with asthma at 6 years of age, stratified by ancestry.

	Caucasian (n=2620)				East Asian (n=352)				P interaction
	Total	**Asthma (%)**	**PR[a] (95% CI)**	**P value**	**Total**	**Asthma (%)**	**PR[a] (95% CI)**	**P value**	
All Infants									
No eczema or food allergy	1,662	151 (9.1)	1.0	-	135	8 (6.0)	1.0	-	-
Food allergy only	92	21 (22.8)	2.45 (1.62-3.71)	<0.001	24	3 (12.5)	1.89 (0.49-7.37)	0.357	0.966
Eczema only	464	63 (13.6)	1.46 (1.11-1.92)	0.007	71	5 (7.0)	1.14 (0.37-3.49)	0.816	0.788
Eczema and food allergy	154	52 (33.8)	3.56 (2.72-4.67)	<0.001	49	12 (24.5)	3.12 (1.21-8.08)	0.019	0.874
Excluding infants wheezing during first year of life									
No eczema or food allergy	1,276	103 (8.1)	1.0	-	105	8 (7.6)	1.0	-	-
Food allergy only	73	15 (20.6)	2.52 (1.55-4.11)	<0.001	20	2 (10)	1.36 (0.28-6.54)	0.699	0.584
Eczema only	326	34 (10.4)	1.28 (0.89-1.85)	0.184	47	0	Omitted[b]	-	-
Eczema and food allergy	117	37 (31.6)	3.8 (2.74-5.28)	<0.001	29	9 (31)	3.5 (1.26-9.69)	0.016	0.983

[a] Prevalence ratio (PR) adjusted for sex, socioeconomic status, family history of allergic disease and antibiotics use

[b] Prevalence ratio could not be calculated because there were no children with asthma in this group

Associations between early life eczema and food allergy status and allergic rhinitis at age 6

Among Caucasian children, those with both eczema and food allergy at age 1 were four-fold more likely to have allergic rhinitis at age 6 compared to those with no eczema and no food allergy at age 1 (PR 4.26, 95% CI 2.97-6.11). Although the magnitude of this association was lower for Asian children (PR 2.29, 95% CI 1.1-4.8), there was only modest evidence that ancestry modified the association between food allergy and eczema status at age 1 and allergic rhinitis at age 6 (P interaction = 0.091, Table 3.3). Food allergy alone was also associated with allergic rhinitis in both Caucasian and East Asian children, whereas eczema alone was only associated with allergic rhinitis in Caucasian children (Table 3.3).

Similar results were observed in the sensitivity analyses (see Tables E1-E3 in the Online Repository), although the magnitude of some of the associations were attenuated.

Table 3.3 Association between food allergy and eczema at 1 year with allergic rhinitis at 6 years of age, stratified by ancestry.

	Caucasian (n=2620)				East Asian (n=352)				
	Total	**Allergic rhinitis (%)**	**PR[a] (95% CI)**	**P value**	**Total**	**Allergic rhinitis (%)**	**PR[a] (95% CI)**	**P value**	**P interaction**
No eczema or food allergy	1,190	76 (6.4)	1.0	-	95	11 (11.6)	1.0	-	-
Food allergy only	69	13 (18.8)	2.81 (1.67-4.74)	<0.001	19	8 (42.1)	4.3 (2.19-8.42)	<0.001	0.688
Eczema only	333	51 (15.3)	2.16 (1.54-3.04)	<0.001	55	13 (23.6)	1.64 (0.75-3.56)	0.213	0.559
Eczema and food allergy	114	34 (29.8)	4.26 (2.97-6.11)	<0.001	41	13 (31.7)	2.29 (1.1-4.8)	0.027	0.091

[a] Prevalence ratio (PR) adjusted for sex, socioeconomic status, family history of allergic disease

3.3.5 Discussion

We have shown through this study that East Asian children have a higher prevalence of most allergic diseases at 6 years of age. Asthma appears to be similar between East Asian and Caucasian children. However, there was no evidence of a differential progression of the atopic march in East Asian children compared with Caucasian children. Children with both IgE-mediated food allergy and eczema during infancy had an increased risk of allergic rhinitis and asthma at age 6, regardless of ancestry. Caucasian children with food allergy or eczema alone also had an increased risk of asthma and allergic rhinitis, although the magnitude of association was lower than those with both food allergy and eczema.

Our finding that asthma appears to follow a different pattern to other allergic diseases in East Asian children was unexpected. Sensitisation to aeroallergens has been shown to be a strong risk factor for wheezing (180) and asthma (181). However, although aeroallergen sensitisation was twice as common in East Asian children, the prevalence of asthma was not similarly increased. Asthma was less common in East Asian children with aeroallergen sensitisation compared with aeroallergen sensitised children of Caucasian parents. A previous study of three South East Asian populations also reported that the prevalence of asthma did not correspond well to prevalence of atopy in these populations – despite differences in the prevalence of asthma between Hong Kong (7%), Kota Kinabalu, Malaysia (3%) and San Bu, China (2%), the prevalence of atopy (defined by positive skin prick test) in these countries was similar (182). In the same study, Leung and Ho also found that family history was a stronger risk factor for asthma and allergic disease than aeroallergen sensitisation in these three populations.

There are several possible explanations for the finding that asthma, unlike other allergic diseases, was not more common among East Asian children. Ethnic differences in lung function have been well-documented across all ages (183-187). Previous studies reported that, compared to Caucasians, South and North East Asians had reduced forced expired volume in 1 sec (FEV_1: a measure of airway calibre) and forced vital capacity (FVC: a measure of lung size) (185), citing inspiratory muscle strength, lung compliance or chest size as possible explanations for the differences (183, 188, 189). Lack of power to detect

a difference is another potential issue, given that the prevalence of asthma was lower than other allergic diseases. Nevertheless, the marked differences observed between the two ancestry groups in terms of sensitisation rates suggest that this is not the sole reason for the findings. Additionally, non-differential misclassification (such as misclassification of viral wheeze as asthma) could reduce the magnitude of the association. Finally, it is possible that East Asian parents are less likely to recognise or seek medical diagnosis of asthma symptoms in their child. However, this latter possibility seems unlikely since the East Asian group was more often diagnosed with other allergic conditions, showing that this group were being seen in the health system. Reasons for this finding warrant further exploration.

Apart from asthma, other allergic diseases (IgE-mediated food allergy, eczema, aeroallergen sensitisation and allergic rhinitis) were very common in East Asian children. It has been proposed that the historical parasite endemic of the previous era shaped the evolution of the immune system in populations residing in tropical regions such as those in East Asia (135). Genetic studies have shown correlations between geographical parasite prevalence and genetic diversity in genes involved in immune defence, and immune disease (190). These signatures of genetic adaptation in the immune system point to regional endemic pathogen load as a strong selective pressure on human evolution. Theoretically, the selection of particular pro-inflammatory genotypes under historically high pathogen load may predispose to an 'over-active' immune profile in modern environments, where many chronic infections have been eliminated and migration is common. This might contribute to the extremely high rates of allergy in East Asian children born into the westernised environment of Australia. It has also been shown that age at migration is an important determinant for prescription of asthma medication, suggesting that environmental exposure in early life are critical (191).

Our findings add to current knowledge about the role of infant food allergy, primarily, IgE-mediated food allergy, separate from and in conjunction with eczema, in the atopic march. Previous studies have predominantly focussed on eczema or food sensitisation in early life, and studies of challenge-proven food allergy are limited (34, 71). In addition, no previous studies have explored potential differences in the atopic march in children with different ethnic backgrounds. We also found several demographic differences, such

as sex, pet ownership and childcare attendance between Caucasians and East Asians, which may have contributed to differences in allergic disease prevalence between these groups. We have adjusted for these factors in our logistic regression models by including them as confounders if the magnitude of association between exposure and outcome changed by more than 10%.

One of the strengths of this study cohort is the high participation rate both at recruitment and at follow-up. Recruited participants are also broadly representative of the general population (156). Furthermore, most food allergy studies are based on self-reported food allergy and an advantage of our study is the use of oral food challenge to confirm food allergy status. Notwithstanding, our study does have some limitations. We found differences in several demographic and other characteristics for those lost to follow-up compared to those who participated in follow-up at age 6 years. To account for this participation bias, we adjusted for these differences using re-weighting, which showed our results remained consistent. It should also be noted that diagnosis of wheeze/asthma remains challenging at age 6. It is likely that some children may have continuing early transient wheeze or infectious induced symptoms. Atopic asthma, which generally becomes persistent, only occurred in a minority of patients (<5%). Moreover the overall prevalence of asthma was low, which limits the likelihood of finding any association to ethnicity. Therefore, the negative finding for asthma require further investigation. Follow-up of this cohort at age 10 years is currently underway and may shed more light on the issue. Our categorisation for East Asians and Caucasians were based on parental country of birth which was used as a proxy for ancestry. While some misclassification is likely using this definition, we have supporting evidence from our own study that self-reported ancestry correlates well with genetically inferred ancestry in this population (130). Additionally, we have grouped all the East Asian countries together to increase statistical power but there might be some differences between countries that we were unable to explore. We were also not powered to look at other Asian groups such as the South Asians. Lastly, the lack of difference in association between food allergy and eczema status at age 1 and asthma diagnosis or allergic rhinitis at age 6 by ancestry may be due to the small sample size of East Asian children in the study.

3.3.6 Conclusion

High rates of allergy among East Asian children in infancy appear to be maintained into early childhood, with a high prevalence of eczema and allergic rhinitis at 6 years of age. Atopic asthma appears to be similar between East Asian and Caucasian children. Our findings identify East Asian children as a high risk allergic group not just in infancy but throughout early childhood. We also showed that IgE-mediated food allergy and eczema in infancy increase the risk of asthma and allergic rhinitis in early childhood, irrespective of ancestry.

Chapter 4 Comparison of food allergy prevalence between Asian populations in Singapore and Melbourne

This chapter is written as a traditional thesis chapter, although a manuscript is currently in preparation. The results of this chapter were presented at an Oral session at the European Academy of Allergy and Clinical Immunology - Food Allergy and Anaphylaxis 2018 meeting, in October at Copenhagen, Denmark.

4.1 Introduction

Food allergy has been implicated in a phenomenon described as the 'second wave' of the allergy epidemic, with rising prevalence observed globally. Although not well studied, it is popularly believed that food allergy prevalence in Western countries is much higher in comparison to prevalence of food allergy in Asian countries (7, 192). While an increasing prevalence has been observed primarily among developed Western countries, several regions in the developing world such as China and Africa are now also seeing an increase in food allergy prevalence (193-199). The increase in prevalence of allergy in Asian countries and/or developing countries appears to be concurrent with increasing westernisation and urbanised lifestyle in these countries. Adoption of Westernised lifestyle and dietary habits along with changes in composition of environment and allergens, may increase the risk of food allergy in these countries (193). Asian migrants living in Western countries may also experience rapid lifestyle and environmental changes on migration from their country of origin. However, few studies have directly compared food allergy prevalence between Asian and Western countries in local or migrant populations using standardised definitions of food allergy.

In this regard, there is mounting evidence on the high rates of food allergy in Asian migrants living in Western countries. In one study, food allergy rates in East Asian infants who were born in Australia and living in Melbourne were three times higher than Australian born Caucasian infants (49). Paradoxically, the parents of these East Asian infants themselves have lower rates of allergic disease compared with Australian-born parents.

On the other hand, children who were born in Asia and migrate to Australia have lower rates of nut allergy than the local population (166). Similar findings have also been observed in the United States and United Kingdom for food sensitisation (21) and food allergy among foreign-born children (22, 200, 201). The sudden rise in prevalence of food allergy in the East Asian population was observed within a single generation and occurred within too short a time to be attributed to genetic factors alone. It is more likely that a genetic predisposition to food allergy conferred by Asian heritage may have been unmasked upon exposure to environmental risk factors in the Western environment. Alternatively, these observations may be a result of the removal of protective factors present in the Asian environment.

One way of untangling contributing risk factors is to compare prevalence of disease and environmental exposure between Asians living in Asia and Asians living in Western country. In order to study potential environmental risk factors for food allergy, the use of well characterised studies with starkly different food allergy rates are helpful. The studies would also need to be similar methodologically. Otherwise, differences in methodological factors would limit the conclusions that could be drawn. The comparison in prevalence and risk factors for food allergy among Asian populations living in different geographical locations have not been previously explored.

Here, we chose to compare the prevalence of food allergy in Asian populations in two geographical regions – Melbourne and Singapore. Singapore is a modern and developed Southeast Asian country and its population disease profile is made up of illnesses such as cancer, metabolic syndrome and allergy (202). Singapore has been reported to have a low food allergy rate, with an overall prevalence of 2.9% in 12 month-old infants (19). On the other hand, Melbourne, also a developed country, is known as the food allergy capital of the world, with a comparatively higher food allergy prevalence of 10% in 12-month-old infants (17), surpassing that of other Western countries (7, 203-206). Using data obtained from two cohort studies originating from Singapore and Melbourne, the aim of this chapter is to compare the prevalence of food allergy in East Asian children born and living in Singapore to those born and living in Melbourne. Additionally, this chapter also assessed potential differences in risk factors for food allergy that might explain any differences in prevalence of food allergy between the two regions.

4.2 Research questions

1. Is there a difference in the prevalence of food allergy between infants with East Asian parents living in Singapore and those living in Australia?
2. What are the risk factors for food allergy in each of the East Asian populations?
3. Does the prevalence of these risk factors differ between Singapore and Melbourne, Australia?

4.3 Methodology

This study used data from the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) birth cohort and a longitudinal study of food allergy in Melbourne, Australia (HealthNuts). Asian infants from GUSTO were recruited from hospitals between 2009-2010 while Asian infants from HealthNuts were recruited from immunisation centres between 2007-2010.

4.3.1 Study cohort

4.3.1.1 HealthNuts study

Details of the recruitment process have previously been described in Chapter 2, Section 2.1.1. In short, baseline data were collected when infants were recruited at community immunisation centres (N=5,276) at age 11-15 months (mean age 12.7 months). Infants underwent skin prick testing to four common allergens (egg, peanut, sesame, shrimp/cow's milk) at the immunisation centres. Infants with a detectable wheal size $\geq$ 1 mm to any of the foods were invited to the HealthNuts clinic at the Royal Children's Hospital, Melbourne for an OFC to ascertain their food allergy status. During the clinic visit, infants underwent repeat skin prick testing and blood testing to determine their food specific IgE level. The infants' mean age at the first clinic visit was 13.9 ± 1.3 months. OFCs were carried out using a pre-determined protocol described earlier in Chapter 2. The following objective criteria were used to define a positive OFC: three or more concurrent, non-contact urticaria persisting for at least 5 minutes, perioral or periorbital angioedema, vomiting or evidence of circulatory or respiratory compromise, occurring

within 2 hours of ingestion of a dose during food challenge. The food challenge was deemed negative if the infant was able to complete the challenge with no reaction (157).

Ethics approval was obtained from the Human Research Ethics Committee (HREC) of the Victorian State Government Office for Children (reference no. CDF/07/492) and Department of Human Services (reference no. 10/07) as well as the Royal Children's Hospital HREC (reference no. 27047). Parents gave written consent for child's participation in the study.

4.3.1.2 GUSTO study – Allergy domain

The GUSTO study is a population-based birth cohort study involving pregnant women aged 18 years and above. The primary objective is to study early life factors in metabolic diseases such as obesity and type 2 diabetes. As an all-encompassing birth cohort, GUSTO has several secondary objectives and domains – one of which is allergy.

Women who i) attended their first trimester antenatal ultrasound scan at one of Singapore's two major public maternity units, the National University Hospital and the KK Women's and Children's Hospital from 2009; and ii) will be residing in Singapore for the next 5 years after recruitment, were eligible for recruitment into GUSTO. However, only women who agreed to donate birth tissues including placenta and cord blood at delivery were included.

All pregnant mothers enrolled in the main GUSTO study were eligible for recruitment into the allergy domain. Enrolment of mothers occurred between June 2009 until September 2010, with a total of 1247 mother-infant pairs of Chinese, Malay and Indian ethnicities (response rate=61.3%) (207).

In the allergy domain, skin prick tests to milk, egg and peanut were done at ages 18, 36 and 60 months. Food allergy data were obtained via a self-reported history of food allergy questionnaire at 6, 12, 18, 36, 48 and 60 months of age. Information on demographics, lifestyle and nutritional data from pregnancy (3rd trimester onwards) and at 6 monthly time-points throughout the child's life were also collected. Nutritional data detailing food types and quantities were collected using a 24-hour recall and a 3-day food diary (208).

Further details on the methodology and recruitment process can be found in Soh et al (202). The ethics boards of both hospitals approved the GUSTO study.

4.3.2 Definitions

4.3.2.1 Ethnicity definitions

Asians in GUSTO consisted of children born in Singapore whose parents and grandparents were of homogenous Chinese, Malay or Indian ethnicity, which are the three main ethnic groups in Singapore.

The parent's country of birth acts as a proxy for ancestry background in HealthNuts. We have shown previously that parent's country of birth information correlated well with genetically inferred ancestry (131). ***Asians in HealthNuts*** consisted of children born in Australia with **both** parents born in North East Asia region such as China, Hong Kong, Japan, Taiwan, Macau, North Korea, South Korea, South East Asia region of Vietnam, Philippines, Singapore, Thailand, Indonesia, Malaysia, Cambodia, Laos and South Asia region of India and Sri Lanka. These countries were grouped based on the SACC which were developed to be applicable to Australia's multicultural society for use in analysing Australian-based country of origin data (161). Groups in the SACC comprise geographically proximate countries which have broadly similar social, cultural, economic and political characteristics.

The definition used to categorise Asians in HealthNuts was not as precise as that used in GUSTO as the HealthNuts study did not collect the exact information on their ethnic groups. In particular if the parent's country of birth is Singapore/Malaysia/Indonesia, it is not possible to untangle if they are of Chinese, Malay or Indian ethnic group. While we do also ask about parent's ethnicity, these are broadly categorised as Caucasian, Asian, African, Aboriginal or Torres Strait Islander or Middle Eastern. Therefore, we chose a population that was as similar as possible to that of GUSTO.

4.3.2.2 Outcome definitions – food allergy

To harmonise food allergy definitions, we created new variables with definitions that were as similar as possible.

In GUSTO, food allergy was defined by:

i) a positive skin prick test (SPT) of ≥ 3 mm to egg, peanut or cow's milk at 18 months **AND**
ii) a convincing history of IgE-mediated reaction upon exposure to the relevant food.

In HealthNuts, food allergy was defined as:

i) positive SPT ≥ 3 mm to egg or peanut and a corresponding positive oral food challenge to egg or peanut at 14-18 months **OR**
ii) positive SPT ≥ 3 mm to cow's milk and a convincing history of reaction to cow's milk. Oral food challenge was not carried out for cow's milk.

4.3.2.3 Exposure variables definitions

We also used harmonised exposure definitions where possible. Details on definitions of each of the exposure variables are listed in Table 4.1.

Table 4.1 Definitions of exposure variables used in HealthNuts and GUSTO studies

Variable	HealthNuts	GUSTO
Childcare attendance	Attendance at day care, childcare or family care (small group childcare in the home of a carer) during first year of life.	Attendance at day care or childcare in the first year of life.
Age at first childcare attendance	Age in months at which child started to attend any type of childcare. Categorical variable was set up with following categories: "no childcare", "childcare before 6 months", "childcare after 6 months".	Age in months at which child began attending any type of day care/childcare. Categorical variable was set up with following categories: "no childcare", "childcare before 6 months", "childcare after 6 months".
Antibiotics use	Categorical variable with an affirmative response to the question "Has your child ever had antibiotics" at 12 months.	Defined as a positive response to the question "Has your child ever had antibiotics?" at any time-point up to 12 months.
Mode of Delivery	Categorical variable defined as either "vaginal" or "caesarean section" delivery.	Categorical variable defined as either "vaginal" or "caesarean section" delivery.
Gestational age at delivery	Categorical variable with the following categories: <37 weeks (pre-term), ≥ 37 weeks.	Categorical variable with the following categories: <37 weeks (pre-term), ≥ 37 weeks.
Birthweight	A continuous variable with child's birth weight in grams.	
Maternal Education	Highest education or vocational qualification completed by child's mother. A categorical variable consisting of "pre-tertiary", "tertiary" and others was created. Mothers who completed Year 12 and below were grouped as having completed "pre-tertiary" education. University degree and postgraduate university	Highest education or vocational qualification completed by child's mother. A categorical variable consisting of "pre-tertiary", "tertiary" and others was created. Mothers who completed GCE or ITE (Institute of Technical Education) education only were grouped as having completed

Variable	HealthNuts	GUSTO
	degree were categorised as "tertiary" and other responses including trade apprenticeship and technical diploma/certificate were categorised as "others".	"pre-tertiary" education. University degree and postgraduate university degree holders were categorised as "tertiary" and other responses including trade apprenticeship and technical diploma/certificate were categorised as "others".
Any Siblings	Categorical variable with an affirmative response to the question "Does your 12 month old have other brothers or sisters?"	Categorical variable defined as other children born to the mother, living in the same household.
Cat Ownership	Presence of a cat at home at the time of completing the questionnaire when the child was 1 year of age.	A positive response to the question "Do you have a cat at home?" at 6 or 12 months questionnaire.
Dog Ownership	Presence of a dog at home at the time of completing the questionnaire when the child was 1 year of age.	A positive response to the question "Do you have a dog at home?" at 6 or 12 months questionnaire.
Paternal, maternal and sibling's history of atopy	History of asthma, allergic rhinitis, or eczema in the mother, father, or sibling as reported by the parent.	Self-reported diagnosis of asthma, allergic rhinitis, or eczema in the mother, father, or sibling?
Maternal smoking	An affirmative response to the question "Did the mother smoke in pregnancy?"	An affirmative response to the question "Did you smoke during pregnancy?" in the maternal questionnaire.
Household smoking	Household smoking was defined by any smoking inside or outside the home. A categorical variable was created based on responses to the question "does anyone smoke inside the home?" or "does anyone smoke outside the home?"	A categorical variable was created based on responses to the question "anyone in the house smokes?" or "does anyone smoke outside the home?"

Variable	HealthNuts	GUSTO
Infant's atopic eczema		
Eczema	Eczema diagnosis was based on an affirmative response to the question "Has your child ever been diagnosed with eczema?" In conjunction with responses to the question "Age when eczema was first diagnosed", a categorical variable with the following categories were created "no eczema", "eczema before 6 months" and "eczema after 6 months".	Parental-reported doctor's diagnosis of eczema at any time point up until 18 months. A categorical variable was also created with the following categories: "No eczema, Eczema before 6 months" and "Eczema after 6 months".
Eczema + steroids use by 12 months	Mothers indicated use of medication, specifically topical steroids to treat itchy rash other than nappy rash. A variable with the following categories was created "no eczema", "eczema with topical steroids" and "eczema with no topical steroids".	Similar to that coded for HealthNuts study, children classified as "no eczema" if indicated so at 3 months, 6 months AND 12 months questionnaire time point. The rest were classified as "eczema with topical steroids" or "eczema with no topical steroids" accordingly if they have marked so at either 3 months, 6 months OR 12 months questionnaire data. Where there is conflicting data among the time points, classified as the "more severe phenotype".
Infant's diet		
Age at introduction of solids	Age at which solid foods were first introduced into the infant's diet. A categorical variable was created with classification as follows: age at introduction of solids classified as ≤ 6 months and >6 months.	Age at which solid foods were first introduced into the infant's diet. A categorical variable was created with classification as follows: age at introduction of solids classified as ≤ 6 months and >6 months.

Variable	HealthNuts	GUSTO
Age at introduction of egg	Age at which egg (soft boiled, scrambled or hard boiled) or any foods containing egg (e.g meringue, cakes, biscuits) were first introduced into the infant's diet. Categories were set up as follows: ≤ 10 months, > 10 months and not yet given by 12 months. We chose this grouping given that our previous work showed that those introducing egg after 10 months had an increased risk of egg allergy (60).	Age at which whole egg was first introduced into the child's diet.
Age at introduction of peanut	Age at which peanut butter was first introduced into the infant's diet. Categories were ≤ 10 months, 10-12 months, "not yet given". Those who were introducing peanut after 12 months were classified as "not yet given".	Age at which peanut was first introduced into the child's diet.
Duration of breastfeeding	Age in months when breastfeeding was reported to have ceased. A categorical variable with the following categories was set up <6 months, 6-11 months, ≥ 12 months.	Age in months when breastfeeding was reported to have ceased completely. See below questions. A categorical variable with the following categories was set up <6 months, 6-11 months, ≥ 12 months.
Type of milk feeding in first 6 months of life	A categorical variable with the following categories were created: "Fully formula fed", "Mixed feeding" and "Fully breastfed" (up to 6 months of age). This variable was created based on responses from the following questions "age started breastfeeding", "age breastfeeding stopped", "still breastfed", "age infant formula bottle feeding started", "age infant formula bottle feeding stopped".	A categorical variable with the following categories were created: "Fully formula fed", "Mixed feeding" and "Fully breastfed" (up to 6 months of age). This variable was created based on responses to questions such as "Please indicate your baby's type of feed in the last 3 months", "Are you still breastfeeding?", "If no, how old was your baby when he/she last had a breastfeed? Age stopped

Variable	HealthNuts	GUSTO
		(months)", "What proportion of milk your baby is receiving is breastmilk?" at each time-point.
Maternal diet during pregnancy		
Egg consumption	Mother reporting consumption of "eggs" during pregnancy. Separate questions were not asked for whole eggs and foods containing eggs.	**YES** if intake of egg consumption reported in the 24-hr recall or 3-day food diaries. This is regardless of frequency or amount consumed. **NO** if no consumption of egg reported in the 24-hr recall or 3-day food diaries.
Peanut consumption	Mother reporting consumption of "peanuts" during pregnancy including roasted, peanut butter and cakes.	**YES** if intake of peanut and peanut butter consumption reported in the 24-hr recall or 3-day food diaries. This is regardless of frequency or amount consumed. **NO** if no consumption of peanut and peanut butter reported in the 24-hr recall or 3-day food diaries.
Nuts consumption	Mother reporting consumption of "any other nuts" during pregnancy including cashews and walnuts. Mothers were instructed to specify the type of nuts consumed.	**YES** if intake of tree nuts including hazelnut spread consumption reported in the 24-hr recall or 3-day food diaries. This is regardless of frequency or amount consumed. **NO** if no consumption of tree nuts including hazelnut spread reported in the 24-hr recall or 3-day food diaries.
Shellfish consumption	Mother reporting consumption of "shellfish" during pregnancy.	**YES** if intake of any shellfish (clam, mussel, oyster) or crustaceans (prawn, crab, crayfish) consumption reported in the 24-hr recall or 3-day

Variable	HealthNuts	GUSTO
		food diaries. This is regardless of frequency or amount consumed. **NO** if no consumption of any shellfish (clam, mussel, oyster) or crustaceans (prawn, crab, crayfish) reported in the 24-hr recall or 3-day food diaries.
Soy consumption	Mother reporting consumption of "soy or soy products" during pregnancy.	**YES** if intake of soya bean and products consumption reported in the 24-hr recall or 3-day food diaries. This is regardless of frequency or amount consumed. **NO** if no consumption of soya bean and products reported in the 24-hr recall or 3-day food diaries.

4.4 Statistical analyses

4.4.1 Demographics and prevalence estimates

Baseline characteristics between the two cohorts were compared using chi-square tests. Student's t-test was carried out to compare continuous variables (e.g birthweight).

The prevalence of food allergy in each study was estimated as the observed proportion with 95% confidence intervals generated using the normal approximation to the binomial distribution.

4.4.2 Risk factors

Logistic regression models were used to analyse the association between exposure variables and food allergy in each of the two Asian populations separately, to obtain odds ratios (OR) and 95% confidence intervals. For this, three sets of hypothesis-driven analyses were carried out:

i. Adjusted model for inclusion of eczema variables and associated confounders

ii. Adjusted model for inclusion of variables linked to hygiene hypothesis and associated confounders

iii. Adjusted model for inclusion of variables linked to infants/maternal diet and associated confounders

Additionally, interaction analyses were carried out to assess whether the relationship between each potential risk factor and food allergy differed between the two cohorts. Regression models were fitted to the combined group of GUSTO and HealthNuts infants, with product terms added to test for interactions between the study cohort and each risk factor by comparing models with and without interaction terms using the likelihood ratio test.

4.5 Results

Details on recruitment and participation rates in the larger GUSTO (19) and HealthNuts (17, 55) studies have previously been described in detail. In summary, 1247 women were recruited into the GUSTO birth cohort with 1152 of those having singletons and were included in the study. At 12 months, 78.3% (n=902) completed the questionnaires. In the HealthNuts study, 7134 participants were invited to participate and 5276 were eventually recruited into the study (response rate 74%).

For analyses carried out in this chapter, data on food allergy status was available on 878 children in GUSTO and 314 children with dual Asian heritage in HealthNuts.

4.5.1 Prevalence of food allergy

Prevalence of food allergy among Asian children in GUSTO was 2.4% (95% CI 1.6 - 3.6%) compared to 15.3% (95% CI 11.7-19.7%) in Asian children in HealthNuts ($p<0.001$) (Figure 4.1).

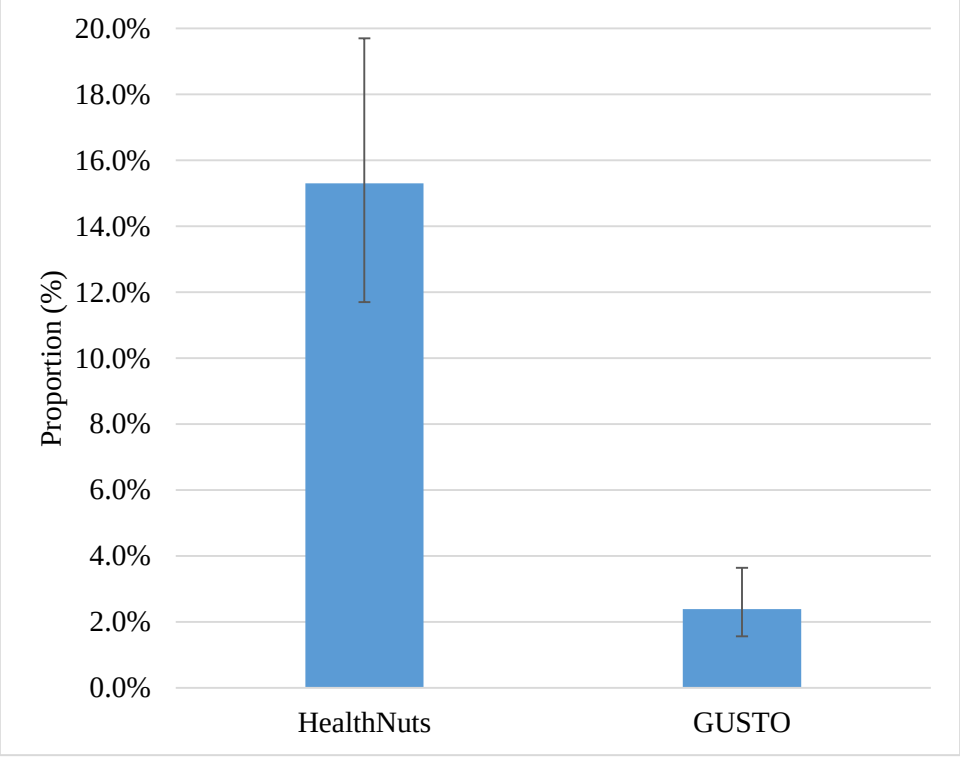

Figure 4.1 Prevalence of food allergy with 95% CI in Asian children in Melbourne HealthNuts (N=314) and Singapore GUSTO cohort (N=878).

4.5.2 Demographics of study cohort

The demographics of participants involved in each study is shown in Table 4.2. The average birthweight of GUSTO participants was lower than HealthNuts participants (p=0.009). Additionally, more GUSTO participants had siblings compared to HealthNuts participants (p<0.001). More of the HealthNuts mothers had completed tertiary education. Family (paternal, maternal and sibling's) history of allergy was more common in HealthNuts participants than GUSTO participants. Other demographic factors (male sex, caesarean section delivery and preterm birth) were similar between the two cohorts.

Table 4.2 Demographics of participants in HealthNuts (N =314) and GUSTO (N=878)

Variables	HealthNuts (%)	GUSTO (%)	p-value
Male sex	169 (54.5)	458 (52.2)	0.476
Caesarean mode of delivery	99 (31.9)	262 (29.9)	0.498
Birthweight, *grams* (mean, range)	3193.8 (1010-4720)	3111.9 (780-4505)	**0.009**
< 37 weeks gestation	17 (6.1)	54 (6.2)	0.951
Maternal education level			
Pre-tertiary (primary or secondary)	12 (6.6)	572 (65.9)	
Tertiary (post-secondary)	140 (76.9)	296 (34.1)	
Others[1]	30 (16.5)	0 (0.0)	< **0.001**
Any sibling	143 (45.5)	510 (62.5)	< **0.001**
Paternal history of atopy	116 (36.9)	192 (21.9)	< **0.001**
Maternal history of atopy	96 (30.6)	192 (21.9)	**0.002**
Sibling history of atopy[2]	59 (41.3)	89 (17.5)	< **0.001**

[1]Others included apprenticeship/trade and diploma qualifications which was unclear if was done at post-secondary or pre-tertiary level.

[2]Sibling's history of atopy among those with siblings only

4.5.3 Environmental exposure

Maternal smoking and household smoking were more prevalent in GUSTO (Table 4.3). Childcare attendance in the first 12 months of life was more common in HealthNuts compared with GUSTO (18.3% compared to 9.6%). However, among those attending childcare, a larger proportion of GUSTO infants started childcare before 6 months whereas more HealthNuts infants started childcare after 6 months (Table 4.3). Rates of pet ownership and use of antibiotics were similar between the two studies.

Table 4.3 Comparison of environmental exposure between Asian infants in HealthNuts (N=314) and GUSTO (N=878)

Exposure	HealthNuts (%)	GUSTO (%)	p-value
Maternal smoking during pregnancy			
No	312 (99.7)	845 (97.6)	
Yes	1 (0.3)	21 (2.4)	**0.018**
Household smoking			
No	251 (80.2)	447 (61.8)	
Yes	62 (19.8)	276 (38.2)	< **0.001**
Age started childcare			
None (ref)	254 (81.7)	651 (90.4)	
Before 6 months	11 (3.5)	44 (6.1)	
After 6 months	46 (14.8)	25 (3.5)	< **0.001**
Cat ownership			
No	304 (96.8)	807 (96.9)	
Yes	10 (3.2)	26 (3.1)	0.956
Dog ownership			
No	290 (92.4)	677 (94.6)	
Yes	24 (7.6)	39 (5.5)	0.176
Antibiotic use			
No	179 (59.1)	413 (54.1)	
Yes	124 (40.9)	351 (45.9)	0.137

4.5.4 Eczema

Table 4.4 compares eczema diagnosis and use of topical steroids as a treatment for eczema in HealthNuts and GUSTO.

Eczema diagnosis was more common in HealthNuts infants than GUSTO infants. Of those with eczema, a higher proportion of HealthNuts infants (84.2%) were using topical steroids compared to GUSTO infants (61.9%), p=0.001.

The percentage of infants with early onset eczema (before 6 months) was also higher in HealthNuts at 30.5% compared to 8.4% in GUSTO, p<0.001.

Table 4.4 Parental report of eczema diagnosis and use of topical steroids as treatment for eczema in HealthNuts (N=314) and GUSTO (N=878).

Exposure	HealthNuts (%)	GUSTO (%)	p-value
Infant eczema			
None (ref)	168 (59.6)	691 (83.2)	
Before 6 months	86 (30.5)	70 (8.4)	
After 6 months	28 (9.9)	70 (8.4)	< **0.001**
Eczema and use of topical steroids			
No eczema (ref)	168 (67.2)	635 (85.8)	
Eczema + steroids	69 (27.6)	65 (8.8)	
Eczema + no steroids	13 (5.2)	40 (5.4)	< **0.001**

4.5.5 Infant's diet

More GUSTO infants had a delayed introduction of solids (> 6 months), egg and peanut into the diet (Table 4.5) compared with HealthNuts. 46.2% of GUSTO infants were not given egg by 12 months compared to only 3.9% of HealthNuts infants, p<0.001. Peanut introduction was delayed in both cohorts, with 80% of HealthNuts infants not given peanut by 12 months and 66.6% GUSTO infants, p<0.001.

Additionally, duration of breastfeeding was shorter among GUSTO infants, with 61.5% of infants breastfed for less than 6 months compared to 33.6% in HealthNuts infants. 87.5% of GUSTO infants were mixed feeding in the first 6 months of life compared to 63% in HealthNuts.

Table 4.5 Age of introduction of solids, allergenic foods (egg and peanut), infant formula feeding and breastfeeding in HealthNuts (N=314) and GUSTO (N=878) infants.

Exposure	HealthNuts (%)	GUSTO (%)	p-value
Age at solid introduction			
≤ 6 months	273 (91.6)	664 (84.1)	
> 6 months	25 (8.4)	126 (16.0)	**0.001**
Age at egg introduction			
≤ 10 months	257 (83.7)	295 (36.5)	
> 10 months	38 (12.4)	140 (17.3)	
Not yet given by 12 mths	12 (3.9)	373 (46.2)	**<0.001**
Age at peanut introduction			
≤ 10 months	40 (13.8)	82 (10.6)	
10-12 months	18 (6.2)	177 (22.9)	
Not yet given by 12 mths	232 (80.0)	516 (66.6)	**<0.001**
Type of milk feeding in the first 6 months of life			
Fully formula fed	12 (3.9)	40 (4.9)	
Mixed feeding	194 (63.0)	710 (87.5)	
Fully breastfed	102 (33.1)	61 (7.5)	**< 0.001**
Duration of breastfeeding			
< 6 months	102 (33.6)	483 (61.5)	
6-11 months	82 (27.0)	133 (16.9)	
≥ 12 months	120 (39.5)	169 (21.5)	**< 0.001**

4.5.6 Maternal diet

Maternal diet practices during pregnancy varied between the two studies for all investigated foods (Table 4.6). Fewer mothers of GUSTO infants consumed nut, peanuts, shellfish and soy during pregnancy compared to mothers of HealthNuts infants (all p<0.001). 56.4% of GUSTO mothers consumed egg during pregnancy but this was still lower compared to HealthNuts mothers where 94.5% consumed eggs during pregnancy (p<0.001).

Table 4.6 Maternal consumption of tree nuts, peanuts, egg, shellfish, crustaceans and soy during pregnancy

Exposure	HealthNuts (%)	GUSTO (%)	p-value
Peanut during pregnancy			
No	41 (14.4)	741 (86.0)	
Yes	244 (85.6)	121 (14.0)	< **0.001**
Egg during pregnancy			
No	16 (5.5)	376 (43.6)	
Yes	277 (94.5)	486 (56.4)	< **0.001**
Tree Nut during pregnancy			
No	44 (17.0)	795 (92.2)	
Yes	215 (83.0)	67 (7.8)	< **0.001**
Shellfish and crustaceans consumption during pregnancy			
No	84 (30.8)	606 (70.3)	
Yes	189 (69.2)	256 (29.7)	< **0.001**
Soy consumption during pregnancy			
No	35 (12.6)	519 (60.2)	
Yes	243 (87.4)	343 (39.8)	< **0.001**

4.5.7 Univariate analyses of risk factors

In the HealthNuts cohort, females were less likely to have food allergy compared to male infants (OR 0.39 (95% CI 0.2-0.77), p=0.008) (Table 4.7). There was also modest evidence of an association between food allergy and sex in the GUSTO cohort (OR 0.43 (95% CI 0.16-1.11); p=0.082)

Family history of allergic disease, including maternal, paternal and sibling, were all associated with an increased risk of food allergy in HealthNuts. In GUSTO, only maternal and sibling history of atopy were associated with an increased risk of food allergy (Table 6). Those with any family history of atopy were at least twice as likely to have food allergy compared to those with no family history of atopy in both cohorts.

Additionally, early onset eczema was associated with food allergy in both studies, p<0.001 for both. The odds of having food allergy for HealthNuts infants with early onset eczema was six times more than those without eczema (OR 6.07 (95% CI 2.95-12.48)). In GUSTO, those with early onset eczema were 7.6 times more likely to have food allergy (95% CI 2.78-20.56); p<0.001). However, the absolute risk of food allergy was lower among those with early onset eczema in GUSTO compared to in HealthNuts. Of those with early onset eczema, only 10% of GUSTO infants also have food allergy compared to 33.7% of HealthNuts infants (p<0.001).

Delayed introduction of egg was a risk factor for food allergy in the GUSTO cohort. GUSTO infants who were not introduced to egg by 12 months were 4 times more likely to have food allergy than infants introduced to egg before 10 months (95% CI 1.17-14.22), p=0.027. In the Asian HealthNuts cohort, there was weak evidence of association between delayed egg introduction and food allergy (OR: 2.88; 95% CI 0.83-10.04; p=0.097)

Infants in the HealthNuts cohort had a lower risk of food allergy at 12 months if mothers consumed eggs or soy during pregnancy (Table 4.7). There was no association observed between infant's food allergy status and maternal egg and/or soy consumption during pregnancy in the GUSTO cohort.

Table 4.7 Univariate analyses of association between environmental exposure and food allergy in HealthNuts and GUSTO

	HealthNuts (N=314)		**GUSTO (N=878)**		**HealthNuts (N=314)**		**GUSTO (N=878)**		**$p_{interaction}$**
	Non Food-Allergic (%)	**Food Allergy (%)**	**Non Food-Allergic (%)**	**Food Allergy (%)**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	
Demographics and environmental factors									
Sex									
Male	134 (51.2)	35 (72.9)	443 (51.7)	15 (71.4)	1	-	1		-
Female	128 (48.9)	13 (27.1)	414 (48.3)	6 (28.6)	0.39 (0.2-0.77)	**0.007**	0.43 (0.16-1.11)	0.082	0.873
Mode of Delivery									
Vaginal	178 (67.9)	33 (68.8)	598 (69.9)	17 (81)	1	-	1		-
Caesarian	84 (32.1)	15 (31.3)	258 (30.1)	4 (19)	0.96 (0.50-1.87)	0.912	0.55 (0.18-1.64)	0.280	0.385
Birthweight	3156 (1010-4720)	3410 (1728-4690)	3108 (780-4505)	3256 (2875-3925)	1 (1-1)	**0.004**	1 (1-1)	0.128	NA
Maternal Education									
Pre-tertiary	11 (7.5)	1 (2.9)	561 (66.2)	11 (52.4)	1	-	1		-
Tertiary (post-secondary	111 (75.5)	29 (82.9)	286 (33.8)	10 (47.6)	2.87 (0.36-23.18)	0.322	1.78 (0.75-4.25)	0.192	0.679
Others	25 (17)	5 (14.3)	0 (0)	0 (0)	2.2 (0.23-21.1)	0.494	NA	NA	NA
Maternal Smoking									
No	264 (99.6)	48 (100)	824 (97.5)	21 (100)	1	-	1		-

	HealthNuts (N=314)		GUSTO (N=878)		HealthNuts (N=314)		GUSTO (N=878)		
	Non Food-Allergic (%)	**Food Allergy (%)**	**Non Food-Allergic (%)**	**Food Allergy (%)**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**$p_{interaction}$**
Yes	1 (0.4)	0 (0)	21 (2.5)	0 (0)	NA	NA	NA	NA	NA
Household Smoking									
No	212 (80)	39 (81.3)	434 (61.4)	13 (81.3)	1	-	1		-
Yes	53 (20)	9 (18.8)	273 (38.6)	3 (18.8)	0.92 (0.42-2.02)	0.842	0.37 (0.11-1.25)	0.111	0.224
Any Siblings									
No	148 (55.6)	23 (47.9)	300 (37.6)	6 (31.6)	1	-	1		-
Yes	118 (44.4)	25 (52.1)	497 (62.4)	13 (68.4)	1.36 (0.74-2.52)	0.324	1 (0.99-1.02)	0.650	0.944
Childcare attendance									
No	220 (82.7)	41 (85.4)	636 (90.3)	15 (93.8)	1	-	1		-
Yes	46 (17.3)	7 (14.6)	68 (9.7)	1 (6.3)	0.82 (0.34-1.93)	0.645	0.62 (0.08-4.79)	0.650	0.811
Age started childcare									
None	215 (81.4)	39 (83)	636 (90.3)	15 (93.8)	1	-	1		-
before 6M	11 (4.2)	0 (0)	43 (6.1)	1 (6.3)	NA	NA	0.99 (0.13-7.64)	0.989	0.986
after 6M	38 (14.4)	8 (17)	25 (3.6)	0 (0)	1.16 (0.5-2.68)	0.727	NA	NA	NA
Cat Ownership									
No	260 (97.7)	44 (91.7)	787 (96.9)	20 (95.2)	1	-	1		-

	HealthNuts (N=314)		GUSTO (N=878)		HealthNuts (N=314)		GUSTO (N=878)		$p_{interaction}$
	Non Food-Allergic (%)	**Food Allergy (%)**	**Non Food-Allergic (%)**	**Food Allergy (%)**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	
Yes	6 (2.3)	4 (8.3)	25 (3.1)	1 (4.8)	3.94 (1.07-14.52)	**0.039**	1.57 (0.2-12.2)	0.664	0.459
Dog Ownership									
No	245 (92.1)	45 (93.8)	663 (94.7)	14 (87.5)	1	-	1	-	
Yes	21 (7.9)	3 (6.3)	37 (5.3)	2 (12.5)	0.77 (0.22-2.72)	0.694	2.56 (0.56-11.68)	0.225	0.235
Any Antibiotic use									
No	157 (61.3)	22 (46.8)	403 (54.1)	10 (52.6)	1	-	1	-	
Yes	99 (38.7)	25 (53.2)	342 (45.9)	9 (47.4)	1.8 (0.96-3.37)	0.065	1.06 (0.43-2.64)	0.900	0.347
Family and personal history of atopy									
Paternal Atopy									
No	180 (67.7)	18 (37.5)	671 (78.3)	15 (71.4)	1	-	1	-	
Yes	86 (32.3)	30 (62.5)	186 (21.7)	6 (28.6)	3.49 (1.84-6.6)	**<0.001**	1.44 (0.55-3.77)	0.454	0.134
Maternal Atopy									
No	196 (73.7)	22 (45.8)	674 (78.6)	12 (57.1)	1	-	1	-	
Yes	70 (26.3)	26 (54.2)	183 (21.4)	9 (42.9)	3.31 (1.76-6.21)	**<0.001**	2.76 (1.15-6.66)	**0.024**	0.744
Family History Atopy									
No	122 (45.9)	11 (22.9)	512 (59.7)	7 (33.3)	1	-	1	-	
Yes	144 (54.1)	37 (77.1)	345 (40.3)	14 (66.7)	2.85 (1.39-5.83)	**0.004**	2.97 (1.19-7.43)	**0.020**	0.945

	HealthNuts (N=314)		GUSTO (N=878)		HealthNuts (N=314)		GUSTO (N=878)		$p_{interaction}$
	Non Food-Allergic (%)	**Food Allergy (%)**	**Non Food-Allergic (%)**	**Food Allergy (%)**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	
Sibling Atopy[1]									
No	72 (61.0)	12 (48)	413 (83.1)	8 (61.5)	1	-	1	-	
Yes	46 (39)	13 (52)	84 (16.9)	5 (38.5)	1.70 (0.71-4.04)	0.233	3.07 (0.98-9.62)	**0.054**	0.416
Eczema Diagnosis									
No eczema	155 (92.3)	13 (7.7)	681 (98.6)	10 (1.4)	1	-	1	-	
Before 6M	57 (66.3)	29 (33.7)	63 (90)	7 (10)	6.07 (2.95-12.48)	**<0.001**	7.57 (2.78-20.56)	**<0.001**	0.725
After 6M	23 (82.1)	5 (17.9)	67 (95.7)	3 (4.3)	2.59 (0.85-7.95)	0.096	3.05 (0.82-11.35)	0.096	0.854
Eczema diagnosis with steroid use									
No eczema	155 (92.3)	13 (7.7)	628 (98.9)	7 (1.1)	1	-	1	-	
Eczema + steroids	45 (65.2)	24 (34.8)	59 (90.8)	6 (9.2)	6.36 (3.00-13.49)	**<0.001**	9.12 (2.97-28.04)	**<0.001**	0.601
Eczema + no steroids	9 (69.2)	4 (30.8)	36 (90)	4 (10)	5.3 (1.43-19.57)	**0.012**	9.97 (2.79-35.62)	**<0.001**	0.497
Infant Diet									
Solid Introduction									
≤ 6 months	234 (91.8)	39 (90.7)	647 (84.0)	17 (85)	1	-	1	-	
>6 months	21 (8.2)	4 (9.3)	123 (16.0)	3 (15)	1.14 (0.37-3.51)	0.816	0.93 (0.27-3.22)	0.907	0.808
Egg Introduction									
≤10 months	219 (84.6)	38 (79.2)	292 (99)	3 (1)	1	-	1	-	
> 10 months	32 (12.4)	6 (12.5)	139 (99.3)	1 (0.7)	1.08 (0.42-2.76)	0.871	0.7 (0.07-6.79)	0.759	0.729

	HealthNuts (N=314)		GUSTO (N=878)		HealthNuts (N=314)		GUSTO (N=878)		
	Non Food-Allergic (%)	**Food Allergy (%)**	**Non Food-Allergic (%)**	**Food Allergy (%)**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**$p_{interaction}$**
not yet given by 12 months	8 (3.1)	4 (8.3)	358 (96)	15 (4)	2.88 (0.83-10.04)	0.097	4.08 (1.17-14.22)	**0.027**	0.700
Peanut Introduction									
≤ 10 months	35 (14.1)	5 (11.9)	81 (98.8)	1 (1.2)	0.77 (0.29-2.12)	0.623	0.48 (0.06-3.7)	0.479	0.675
> 10 months	17 (6.9)	1 (2.4)	175 (98.9)	2 (1.1)	0.32 (0.04-2.48)	0.276	0.44 (0.1-1.98)	0.286	0.803
not yet given by 12 months	196 (79)	36 (85.7)	503 (97.5)	13 (2.5)	1	-	1	-	
Type of milk feeding									
Fully formula feed	9 (3.5)	3 (6.4)	39 (4.9)	1 (5)	1	-	1	-	
Mixed feeding	165 (63.2)	29 (61.7)	696 (88)	14 (70)	0.53 (0.13-2.06)	0.358	0.78 (0.1-6.12)	0.817	0.752
Fully breastfed	87 (33.3)	15 (31.9)	56 (7.1)	5 (25)	0.52 (0.13-2.13)	0.362	3.48 (0.39-30.98)	0.263	0.151
Duration of breastfeeding									
<6 months	85 (33.0)	17 (37)	474 (61.9)	9 (47.4)	1	-	1	-	
6 to <12 months	69 (26.7)	13 (28.3)	130 (17.0)	3 (15.8)	0.94 (0.43-2.07)	0.882	1.22 (0.32-4.55)	0.772	0.746
≥ 12 months	104 (40.3)	16 (34.8)	162 (21.2)	7 (36.8)	0.77 (0.37-1.61)	0.487	2.28 (0.83-6.21)	0.108	**0.088**
Maternal Diet during pregnancy									
Peanut consumption									

	HealthNuts (N=314)		GUSTO (N=878)		HealthNuts (N=314)		GUSTO (N=878)		$p_{interaction}$
	Non Food-Allergic (%)	**Food Allergy (%)**	**Non Food-Allergic (%)**	**Food Allergy (%)**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	
No	33 (13.7)	8 (18.2)	723 (85.9)	18 (90.0)	1	-	1	-	
Yes	208 (86.3)	36 (81.8)	119 (14.1)	2 (10.0)	0.71 (0.31-1.67)	0.437	0.68 (0.15-2.95)	0.601	0.949
Tree nuts and other nuts consumption									
No	36 (16.4)	8 (20)	775 (92.0)	20 (100)	1	-	1	-	
Yes	183 (83.6)	32 (80)	67 (8.0)	0 (0.0)	0.79 (0.34-1.85)	0.582	NA	NA	NA
Egg consumption									
No	10 (4)	6 (13.6)	364 (43.2)	12 (60.0)	1	-	1	-	
Yes	239 (96)	38 (86.4)	478 (56.8)	8 (40.0)	0.26 (0.09-0.77)	**0.015**	0.51 (0.21-1.25)	0.142	0.363
Shellfish consumption[2]									
No	66 (28.8)	18 (40.9)	609 (72.3)	14 (70.0)	1	-	1	-	
Yes	163 (71.2)	26 (59.1)	233 (27.7)	6 (30.0)	0.58 (0.30-1.14)	0.114	1.01 (0.39-2.67)	0.976	0.358
Soy consumption									
No	25 (10.6)	10 (24.4)	506 (60.1)	13 (65.0)	1	-	1	-	
Yes	212 (89.5)	31 (75.6)	336 (39.9)	7 (35.0)	0.37 (0.16-0.83)	**0.017**	0.81 (0.32-2.05)	0.658	0.209

NA- not applicable. Where no infants fall into one particular group of a variable (n=0), ORs were not able to be obtained and rows are indicated as NA.
[1]Only among those with siblings
[2]Includes consumption of crustaceans

4.5.8 Adjusted models for analyses of risk factors

A hypothesis-driven approach was carried out in our adjusted models for food allergy. In the first model, we assessed the association between age of eczema diagnosis and risk of food allergy. Age of eczema diagnosis was associated with an increased risk of food allergy in both studies (Table 4.8). Infants with early eczema diagnosis (<6 months) were almost 5 times more likely to also have food allergy compared to those with later diagnosis, in both HealthNuts (aOR 4.86 (2.21-10.71); p<0.001) and GUSTO (aOR 4.93 (1.49-16.34); p=0.009). This association did not seem to differ by study ($p_{interaction}$=0.811).

In our hygiene hypothesis model, cat ownership was associated with an increased risk of food allergy in the HealthNuts study (aOR 5.35 (1.17-24.37); p=0.03). There was no evidence of association between cat ownership and food allergy in the GUSTO study, although the direction of effect is similar to that in HealthNuts (aOR 3.15 (0.36-27.53); p=0.299). The association between cat ownership and food allergy also did not differ by study ($p_{interaction}$=0.607).

Interestingly, when assessing for association between diet and food allergy, none of the exposures were associated with food allergy in both HealthNuts and GUSTO. It is worth nothing however that there is a trend of delayed egg introduction towards an increased risk of food allergy (Table 7). Similarly, this association did not differ by the study population ($p_{interaction}$=0.605).

Table 4.8 Adjusted models for the risk factors for food allergy in HealthNuts and GUSTO.

Food allergy	**HealthNuts (n=278)**		**GUSTO (n=693)**		
Model 1[a]: Eczema	**aOR (95% CI)**	**p-value**	**aOR (95% CI)**	**p-value**	**$p_{interaction}$**
Age of eczema diagnosis					
No eczema	1	-	1	-	
< 6 months	4.86 (2.21-10.71)	**<0.001**	4.93 (1.49-16.34)	**0.009**	0.811
≥ 6 months	2.31 (0.72-7.43)	0.158	3.39 (0.84-13.59)	0.085	0.621
Dog ownership	0.57 (0.13-2.4)	0.442	1.6 (0.32-8.01)	0.565	0.305
Cat ownership	2.79 (0.59-13.25)	0.197	1.81 (0.21-15.52)	0.588	0.772
Female sex	0.37 (0.17-0.78)	**0.009**	0.37 (0.12-1.19)	0.095	0.96
Maternal history of atopy	1.52 (0.72-3.22)	0.271	2.57 (0.89-7.4)	0.08	0.362
Paternal history of atopy	2.54 (1.23-5.26)	**0.012**	1.91 (0.66-5.55)	0.234	0.785

aOR – adjusted odds ratio

[a] all variables listed in this model were included simultaneously and are therefore adjusted for each other

Food allergy	**HealthNuts (n=294)**		**GUSTO (n=600)**		
Model 2[b]: Hygiene hypothesis	**aOR (95% CI)**	**p-value**	**aOR (95% CI)**	**p-value**	**$p_{interaction}$**
Use of antibiotics	1.44 (0.74-2.82)	0.283	1.51 (0.49-4.67)	0.478	0.992
Dog ownership	0.75 (0.2-2.82)	0.67	4.65 (0.92-23.48)	0.063	0.131
Cat ownership	5.35 (1.17-24.37)	**0.03**	3.15 (0.36-27.53)	0.299	0.607
Childcare attend-ance	0.89 (0.36-2.25)	0.81	0.71 (0.09-5.77)	0.746	0.799
Female sex	0.43 (0.21-0.87)	0.019	0.32 (0.08-1.18)	0.087	0.765
Any siblings	1.3 (0.66-2.54)	0.444	2.43 (0.63-9.38)	0.198	0.513
Household smoking	1.03 (0.45-2.35)	0.95	0.33 (0.08-1.27)	0.107	0.226
Caesarean mode of delivery	1.06 (0.52-2.13)	0.877	0.77 (0.2-2.93)	0.704	0.732

aOR – adjusted odds ratio

[b] model adjusted for sex as a confounding factor

Food allergy	**HealthNuts (n=180)**		**GUSTO (n=431)**		
Model 3[c]: Diet	**aOR (95% CI)**	**p-value**	**aOR (95% CI)**	**p-value**	**$p_{interaction}$**
Age of solid introduction					
≤ 6 months	1	-	1	-	

> 6 months	0.79 (0.15-4.12)	0.778	0.53 (0.06-4.49)	0.557	0.767
Age of egg introduction					
≤ 10 months	1	-	1	-	
10-12 months	1.59 (0.43-5.87)	0.485	NA	NA	NA
Not yet given	1.89 (0.36-9.84)	0.449	3.08 (0.62-15.18)	0.168	0.605
Age of peanut introduction					
Not yet given	1	-	1	-	
≤ 10 months	0.79 (0.19-3.28)	0.741	0.64 (0.07-5.98)	0.692	0.909
> 10 months	NA	NA	0.49 (0.09-2.61)	0.405	0.988
Duration of breastfeeding					
< 6 months	1	-	1	-	
6 to 12 months	0.44 (0.12-1.55)	0.199	0.59 (0.06-5.29)	0.634	0.97
≥ 12 months	0.51 (0.18-1.46)	0.21	1.58 (0.42-5.99)	0.501	0.198
Maternal consumption of egg during pregnancy	0.67 (0.1-4.48)	0.677	0.33 (0.09-1.19)	0.089	0.783
Maternal consumption of soy during pregnancy	0.3 (0.07-1.26)	0.099	1.25 (0.37-4.28)	0.718	0.285
Maternal consumption of nuts during pregnancy	0.5 (0.15-1.73)	0.275	NA	NA	NA
Age of eczema diagnosis					
No eczema	1	-	1	-	
< 6 months	3.65 (1.3-10.26)	0.014	3.64 (0.74-18)	0.113	0.96
≥ 6 months	1.79 (0.38-8.34)	0.46	2.68 (0.48-14.83)	0.259	0.991
Maternal history of atopy	2.38 (0.91-6.24)	0.077	2.22 (0.6-8.24)	0.233	0.84
Paternal history of atopy	2.45 (0.94-6.42)	0.068	0.88 (0.2-3.81)	0.865	0.31
Female sex	0.36 (0.13-1)	0.049	0.65 (0.18-2.32)	0.507	0.489

aOR – adjusted odds ratio, NA – not applicable. Odds ratios could not be obtained as there were no infants in that particular group. For example, in model 3, for egg introduction, there were no infants introduced to egg between 10 to 12 months with food allergy in the GUSTO study.

[c] model adjusted for confounders sex, eczema diagnosis and family history

4.6 Discussion

In this chapter, we explored the prevalence and risk factors for food allergy between two Asian populations in Singapore and Melbourne. The prevalence of food allergy between the two Asian populations was significantly different, with prevalence in HealthNuts, Melbourne at 15.3% and in GUSTO, Singapore at 2.4%. One of the key findings was that more HealthNuts infants were also diagnosed with eczema than GUSTO infants. Among those with eczema, early onset and more severe eczema was also more common in Melbourne. Early onset eczema was associated with an increased risk of food allergy in both HealthNuts and GUSTO and the observed association did not differ by study.

There were also dietary differences between the two studies. A large majority of GUSTO infants had egg introduced into their diet after 10 months. Despite delayed introduction of egg into the diet, GUSTO reported a lower prevalence of food allergy. The vast majority of infants in both studies had not consumed peanut by 12 months, although the proportion delaying to after 12 months was slightly higher in GUSTO. There was limited evidence of an association between age at peanut introduction and food allergy risk, potentially due to lack of statistical power. Exclusive breastfeeding was shorter in GUSTO but this was not associated with food allergy risk. Apart from infant's diet, there were also noteworthy differences in the maternal diet during pregnancy. Only a small proportion of GUSTO mothers were consuming common allergenic foods (peanut, soy, egg, nuts, shellfish) during pregnancy compared to HealthNuts mothers.

In addition, several demographic and environmental factors differed between the two studies. Family history of allergy and maternal tertiary education were more common in HealthNuts than GUSTO. There were also higher maternal and household smoking as well as larger families in GUSTO. The differences in these factors may be due to cultural and societal influences, as well as maternal educational background and awareness of prevailing infant feeding guidelines.

Surprisingly, cat ownership was associated with an increased risk of food allergy in the HealthNuts East Asian children. However, the CIs were wide and cat ownership was low within the population (3.2%). Regardless, this finding was unexpected given that previous studies carried out in the whole cohort of HealthNuts study did not identify an association

between food allergy and cat ownership. Instead, dog ownership was found to have a protective effect on risk of egg allergy (55).

4.6.1 Eczema

The finding that eczema is associated with food allergy in HealthNuts is not surprising. Previous studies have established eczema as a well-known strong risk factor for food allergy (71, 167, 209). Our HealthNuts study previously found that one in five infants with eczema also had food allergy (71). Infants with eczema were 11 times more likely to have peanut allergy than those without eczema. The severity and age of onset of eczema have also been shown to significantly increase the risk of food allergy. Half (50.8%) of infants who had eczema within the first 3 months of life and required treatment with topical corticosteroids developed food allergy (71). It was however, interesting to find that more HealthNuts infants have eczema than GUSTO infants. This may be a key driving factor of higher food allergy prevalence in HealthNuts than in GUSTO.

There are two possible reasons for the co-association of eczema and food allergy. Firstly, these findings are consistent with the dual allergen hypothesis where infants who have a weakened skin barrier, such as those with eczema, are at an increased risk of food sensitisation (68), via cutaneous exposure prior to ingestion. The increase in cutaneous exposure to food allergens puts the children at risk of food allergy if foods are not introduced at an early age to induce tolerance. This is particularly relevant to both GUSTO and HealthNuts, where majority of the infants had delayed introduction of food. Secondly, there may be shared genetic (40, 41) and/or environmental risk factors between the two atopic predispositions. Of significance is *FLG* which is commonly associated with eczema (210-212). The loss of function of *FLG* variants have also been implicated in food allergy, specifically peanut allergy (123, 213, 214). *FLG* variants are thought to impair the epidermal barrier, allowing exposure to irritants and allergens, in line with the dual allergen hypothesis. Previously in another study, we found that *FLG* did not have a differential effect on food sensitisation and food allergy (126). There was no significant difference in *FLG* mutation when food sensitised tolerant infants were compared with those with food allergy. This suggests that *FLG* mutations are not essential in the progression

from food sensitisation to food allergy and that there are possibly other factors contributing to the conversion from sensitised tolerant to clinical reactivity.

The latest available study by the International Study of Asthma and Allergies in Childhood (ISAAC) Phase 3 (215) indicated the Asia Pacific region as an area of increasing eczema prevalence (216). While the general prevalence of eczema is high in the region, comparison between the two studies in this chapter showed that eczema prevalence in Singapore is still much lower than Melbourne. Therefore, it is plausible that the lower rate of food allergy in Asians living in Singapore may be due to the lower rate of early onset eczema in the country.

4.6.2 Diet

Despite delayed (>10 months of age) introduction of egg, peanut and shellfish into the diet, GUSTO reported a lower prevalence of food allergy. There was however limited evidence of association between delayed introduction of allergenic food and food allergy in both GUSTO and HealthNuts. This finding is unexpected given that we demonstrated in our previous observational study that infants first exposed to cooked egg at age 4 to 6 months had lower risk of egg allergy compared to first introduction as baked egg (60). This set of analyses were however carried out in the whole HealthNuts population without taking ethnicity into consideration. In contrast, GUSTO previously reported that there was no association between delayed introduction and food allergies, even within the eczema sub-cohort (19).

Nonetheless, there is strong evidence based on randomised controlled trials and other studies that early introduction of allergenic food is protective for food allergy (85). For this reason, the peak professional body of clinical immunology and allergy in Australia and New Zealand, Australasian Society for Clinical Immunology and Allergy (ASCIA) has recommended that infants be introduced to solid food around 6 months but not before 4 months (217). For allergenic food such as egg and peanut, introduction into the diet is recommended within the first year of life.

On the other hand, in Asia, there is a paucity of studies that investigate the association between timing of introduction of food and food allergy. The Asia Pacific Association of

Pediatric Allergy, Respirology & Immunology (APAPARI) therefore released a consensus statement on the collection of evidence for early introduction of food in Asian countries (218). In line with the World Health Organisation (WHO) guideline, healthy infants are recommended to be introduced to complementary food at 6 months. For at-risk infants with family history of atopy, delay is not recommended for allergenic foods (218, 219). Finally, separate approaches befitting of the country's healthcare system should be employed when recommending introduction of allergenic food in infants with severe eczema. In countries with ready access to allergy specialists, referral to allergy specialists for diagnosis should be carried out followed by introduction of allergenic food if indicated necessary (218).

In terms of maternal diet, current recommendations by ASCIA and the American Academy of Paediatrics (AAP) do not recommend excluding common allergenic foods during pregnancy or breastfeeding (220, 221). Previously, strict avoidance of peanuts during pregnancy was recommended by the AAP based on results from a randomised clinical trial that showed reduction of food sensitisation and food allergy during the first year of life following avoidance of allergenic foods during pregnancy. Emerging evidence in the following years suggested against this recommendation and the guideline was changed. A meta-analysis on food allergies and maternal allergen avoidance during pregnancy and/or lactation further supported the new guidelines (222, 223). Despite this, a larger proportion of GUSTO mothers do not routinely consume allergenic foods during pregnancy. This practice may be due to personal preferences or perceived prevention of infant food allergy, both of which we could not confirm as we do not have available data on their behavioural motives.

4.6.3 Meteorological factors

Several ecological studies have implicated macro-level factors such as humidity, vitamin D, climate, season of birth and air pollution in the development of food allergy (102, 224-227). In particular, vitamin D insufficiency has been shown to be a risk factor for food allergy at 12 months (102). In the same vein, a number of studies have found evidence for a relationship between latitude and food allergy prevalence (13, 101, 228). This seems to suggest that allergy prevalence rates are dependent on geographical conditions which

are linked to vitamin D metabolism and production through UVR exposure. Furthermore, admissions to hospital for food allergy-related events, prescriptions of adrenaline auto-injectors for anaphylaxis and prescriptions of hypoallergenic infant formula, all of which are proxy measures of food allergy prevalence, were the highest in regions furthest from the equator (13, 56, 100, 229). The finding that higher rates of diagnosed food sensitisation and allergy were higher in infants born in fall or winter compared with months of more sun exposure in Europe (230), the United States (224), and Australia (100) further substantiate this hypothesis.

We were unable to directly compare infant vitamin D levels in this study but hypothesised that this may contribute to the difference in prevalence of food allergy. Data obtained from the WHO showed a stark difference in UVR exposure between Singapore and Melbourne. In a given year, Singapore's UVR is in the extreme range (UV index 10-13) throughout the year whereas Melbourne's UVR fluctuates according to the different seasons, with the lowest UVR in winter (UV index 2) between June and August and highest in December (UV index 9) (231). It is thus possible that vitamin D status relating to UV exposure may modulate the differences in food allergy status between Asian children living in Singapore and Melbourne, although direct comparisons were beyond the scope of this study.

Another potentially important factor is climate and humidity which has been shown to have a differential effect on eczema based on the climate. The climate and particularly humidity might affect skin moisture which may have an effect on eczema and thereby subsequent development of food allergy. A study carried out in Australian children showed that prevalence of eczema is lower in regions with high sun exposure and warmer climate such as those that are closer to the equator (101). While this finding was replicated by another large study of 0-17 year old children in the United States (232), mixed findings were reported in a study of German children who were observed over 6 months from spring and summer to autumn (233). In the latter study, 21 of the children observed had eczema exacerbations which were alleviated following higher temperatures. The remaining 18 children in the study had symptoms during summer months which were aggravated with higher temperatures. Humidity however had no significant effect on severity of symptom in this study.

Potentially, there may also be a difference in preventing onset of eczema as opposed to exacerbating existing eczema. Poorly controlled eczema has been shown to be associated with geographic areas with increased temperature, sun exposure (total, UVA, and UVB), and humidity (234). According to Langan et al (235, 236), the increased sweating due to the higher humidity may have an irritant effect on the skin due to its acidic pH. This irritation can then activate Th2 and Th17-mediated inflammation which act to downregulate the expression of *FLG* (237, 238). Although the humidity may promote skin moisture, ironically, it also promotes the evaporation of water on the skin surface, further drying the skin (234).

It is still not yet clear how climate, temperature and humidity can affect atopic dermatitis and if indeed the lower prevalence of eczema (and therefore subsequent food allergy) in Singapore is due to higher humidity. It is likely that there are different phenotypes of eczema that exist and are influenced by the temperate or tropical climate in a contrasting manner.

4.6.4 Strengths and limitations

A major strength of this study is the use of well-characterised and comprehensive cohorts. Food allergy diagnosis in HealthNuts is based on the gold standard OFC and prospectively-defined criteria for a positive challenge. As a birth cohort, GUSTO had detailed information taken at several time points within the first year of life which was less susceptible to recall bias.

Nonetheless, it should be noted that there are key differences between the two study cohorts. In particular, mothers in the GUSTO study were recruited in their first trimester of pregnancy, with a study design and purpose that is not central to food allergy. On the contrary, HealthNuts participants were recruited at 12-months and the study design and data collected were centred around food allergy as a primary outcome. Due to inherent differences in study design, collected data on environment exposure and definition of an Asian population were slightly different. We have tried to minimise these differences by using a harmonised definition and a population that was as similar as possible between each study. Apart from that, GUSTO had SPT done at 18 months, whereas in HealthNuts initial SPT in the community was carried out at 12 months when egg allergy might be

higher. However, there is a time lag between recruitment and first clinic visit. When the HealthNuts participants are due for their first clinic visit, the children are between 14 to 16 months and so minimising the difference in age between the two studies. Furthermore, food allergy in HealthNuts was based on oral food challenge whereas in GUSTO diagnosis of food allergy was based on history of reaction and SPT. The difference in definition of food allergy may result in misclassification of food allergy subjects in our analysis. However, there is evidence that history of reaction alongside SPT had a 95% positive predictive value for clinical reactivity, indicating that SPT alongside a history of reaction is a good predictor for food allergy (239, 240) Lastly, our analysis is undermined by the small sample size of the Asian population in HealthNuts and food allergy subjects in GUSTO. We may be insufficiently powered to detect an association for some of the key contributing risk factors. Despite these limitations, it is unlikely that these differences in phenotyping and study design would fully account for the stark contrast in food allergy prevalence. Unmeasured variables might also be contributing to the difference in food allergy rates.

4.7 Conclusions

To sum up, the prevalence of food allergy between two East Asian populations in two geographically different locations were found to be strikingly different. The prevalence of food allergy in East Asians living in Melbourne was 6 times higher than in East Asians living in Singapore. There was also a stark difference in rates of eczema between the two studies, and the lower eczema rate in Singapore may contribute to the lower rate of food allergy in this region. Despite some differences in the study design of the two cohorts, the difference in prevalence seems unlikely to be attributed to these differences alone. The risk factors studied here are unlikely to fully explain the difference in food allergy prevalence. Further study into other factors such as diet, microbiome and meteorological influences may be required to fully elucidate the underlying cause of differential risks of food allergy in the two population groups in Singapore and Melbourne.

Chapter 5 Genetic risk factors for food allergy: A systematic review

This chapter is presented in the form of a manuscript which was submitted for publication in the Allergy Journal on August 2018. The results of this chapter was also presented at an oral session at the European Academy of Allergy and Clinical Immunology - Food Allergy and Anaphylaxis 2018 meeting, in Copenhagen Denmark.

5.1 Study rationale

Complex diseases are often underpinned by multiple common genetic variants which contribute to disease susceptibility. Genetic studies on asthma, allergic rhinitis and eczema have been promising, with several genes of interest identified with confidence despite their limitations. Unlike asthma which has been the focus of genetic research, little has been done in food allergy. The aim of this chapter is to systematically collate existing studies that have investigated the association between genetic factors and food allergy.

5.2 Research questions

1. Based on current literature, which genes have been studied in relation to food allergy and what is the evidence for an association between genetic polymorphisms and food allergy?

5.3 Manuscript: Genetic determinants of paediatric food allergy: A systematic review

Short title: Genetic basis of food allergy: A systematic review

Noor H. A. Suaini[1,2], Yichao Wang[1,2], Victoria Soriano[1,2], David J. Martino[1,2,3], Katrina J. Allen[1,2,4,5], Justine A. Ellis[1,6,7], Jennifer J. Koplin[2,8]

Affiliations:

[1]Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia

[2]Centre for Food and Allergy Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[3]Telethon Kids Institute, University of Western Australia, Perth, Australia.

[4]Department of Allergy and Clinical Immunology, Royal Children's Hospital, Parkville, Victoria, Australia

[5]Institute of Inflammation and Repair, University of Manchester, Manchester, United Kingdom.

[6]Genes, Environment & Complex Disease, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[7]Centre for Social and Early Emotional Development, Faculty of Health, Deakin University, Burwood, Victoria, Australia.

[8]School of Population and Global Health, University of Melbourne, Parkville, Victoria, Australia

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Abbreviations:

CNV: Copy Number Variations

CNVR: Copy Number Variations Region

GWAS: Genome Wide Association Study

SNP: Single Nucleotide Polymorphisms

UTR: Untranslated Region

Conflicts of interest:

The authors declare that they have no conflicts of interest.

Author contributions:

NS, JK and JE were responsible for developing the protocol, search strategy and risk of bias assessment. NS and YW reviewed all titles and abstracts for eligibility against a pre-determined set of inclusion criteria. NS reviewed the full text of potentially eligible papers and extracted data from the original papers, including carrying out the quality assessment of included studies. VS checked the accuracy and authenticity of data extracted. DM and KA contributed to the data analysis and interpretation of data. All authors contributed to the drafting and revising the article for intellectual content and approve the final version of the manuscript.

5.3.1 Abstract

Background

The genetic determinants of food allergy have not been systematically reviewed. We therefore systematically reviewed the literature on the genetic basis of food allergy, identifying areas for further investigation.

Methods

We searched three electronic databases (Medline, Embase and PubMed) through to 9th January 2018. Two authors screened retrieved articles for review according to inclusion criteria and extracted relevant information on study characteristics and measures of association. Eligible studies included those that reported an unaffected non-atopic control group, had genetic information, and were carried out in children.

Results

Of the 2088 studies retrieved, 32 met our inclusion criteria. Five were genome-wide association studies and the remaining were candidate gene studies. 22 of the studies were carried out in a predominantly Caucasian population with the remaining 10 from Asian-specific populations or unspecified ethnicity. We found *FLG, HLA, IL10, IL13,* as well as some evidence for other variants (*SPINK5, SERPINB, C11orf30*) that are associated with food allergy.

Conclusions

Little genetic research has been carried out in food allergy, with *FLG, HLA* and *IL13* being the most reproducible genes for an association with food allergy. Despite promising results, existing genetic studies on food allergy are inundated with issues such as inadequate sample size and absence of multiple testing correction. Few included replication analyses or population stratification measures. Studies addressing these limitations along with functional studies are therefore needed to unravel the mechanisms of action of the identified genes.

5.3.2 Introduction

Food allergy is a complex multifactorial disease with both environmental and genetic risk factors thought to contribute to its pathogenesis. It elicits abnormal immunological reaction upon exposure to certain food proteins, resulting in adverse clinical reactions, most severely anaphylaxis, which can be life-threatening (241).

Existing twin and family studies have shown that genetic composition may play a significant role in the development of food allergy (108, 113, 114). In these studies, genetic differences contribute about 15% to 35% of the observed individual differences in food-specific IgE (108). Twin studies found that monozygotic twins recorded higher concordance rates for sensitisation to peanut allergen than dizygotic twins (113, 114). Sicherer et al found that the heritability estimate for peanut allergy was 82% to 87% (113), demonstrating the role of genetic influence as those with more similar genes (monozygotic twins) were likely to have a more similar phenotype.

The prevalence of food allergy in infants and children below 5 years old appears to be higher in Western countries, compared with Asian countries (242) . However, Australian-born children of Asian parents have a higher prevalence of food allergy compared with both Asian children born in Asia and Australian-born Caucasian children (49, 166). This suggests that the effect of genetic predisposition on food allergy may differ depending on environmental exposures in early life.

Both candidate gene and genome-wide association studies (GWAS) have attempted to identify genes associated with food allergy. An increasing number of GWAS are being carried out primarily for ‘any food allergy’ and peanut allergy outcomes, identifying novel genes associated with these allergies. However, these studies were predominantly in Caucasian or European populations. Candidate gene studies have targeted immune-related genes postulated to be involved in the mechanisms of food allergy. Additionally, given that there are shared genetic risk factors among asthma, allergic rhinitis and eczema (243, 244), there has been work to examine genes previously associated with other allergic diseases for an association with food allergy. However, compared to other allergic diseases, the genetic basis of food allergy remains relatively under-explored. The main

objective of this systematic review is to examine the evidence for the association between genetic polymorphisms and food allergy and identify areas that need further investigation.

5.3.3 Methods

This systematic review was conducted according to a previously developed protocol registered on the international prospective register of systematic reviews (PROSPERO) and reported according to the PRISMA checklist (245).

Search methods for identification of studies

Electronic searching

We searched three databases: Medline (Ovid), Embase (Ovid) and PubMed for references using MeSH terms and thesaurus/keywords on 9th January 2018. PubMed was searched only using keywords to retrieve electronic publications and papers not yet indexed in Medline or Embase. Results were limited to English language and studies of children 0-18 years old. The search strategy was formulated with the help of an experienced librarian at the Royal Children's Hospital and was first developed in Medline (Ovid) and adapted in other databases. The complete search terms and strategies used are listed in the Online Repository Tables S1-S2.

We additionally hand-searched reference lists of reviews and meta-analyses to include any citations that contained information on genetic association of food allergy not captured by the above strategy.

Inclusion criteria of studies

Type of studies

We included cross-sectional studies, case-control studies, prospective, retrospective longitudinal studies (cohorts, case-control studies), family linkage studies, sibling-pair studies, and randomised control trials in our search strategy.

However, only studies that fulfilled the following criteria were included in our final review:

- Presence of unaffected non-atopic control groups in study design

- Study was carried out in children. Studies that spanned childhood and adulthood were also included.

- Studies examined association between food allergy and single nucleotide polymorphisms (SNPs), haplotypes or copy number variants (CNVs).

Case reports and case series were excluded. These often described rare mutations among individual patients with food allergy. Systematic reviews, meta-analyses, conference abstracts, non-original articles (comments, editorials, book chapters) and animal studies were also excluded. Studies carried out in patients with other pre-existing diseases (such as those with food protein-induced enterocolitis syndrome, autism, eosinophilic esophagitis or any other conditions) apart from food allergy were also excluded.

Type of outcomes

The main outcome of the systematic review is clinical food allergy. Studies were included if food allergy diagnosis was determined by an i) oral food challenge or ii) a combination of positive skin prick test and/or specific IgE levels and information on history of food allergy.

Quality assessment

Study quality was assessed by a points scoring system comprising of study reproducibility, study design and statistical analyses, adapted from previous studies (246, 247). These studies based their quality assessments on published checklist and recommendations on replicating genotype-phenotype associations (248) and design of genetic studies in complex diseases (249). Risk of bias was assessed as a measure of study quality but was not used as a basis for inclusion or exclusion of studies. Full details on the criteria for quality assessment and scoring system are included in the Online Repository Table S3.

Data collection and synthesis

Two reviewers (NS and YW) independently screened the title and abstracts of all retrieved citations against the pre-determined inclusion and exclusion criteria. Where there was a discrepancy in labelling of included studies, the full text was reviewed by the same

reviewers. Eligible papers were scrutinized to extract relevant data and assessed for study quality by two reviewers (NS and VS).

Data were extracted from each paper and compiled for each gene. We reported odds ratios with 95% confidence intervals and where available, p-values for association with food allergy as reported by the original paper.

We chose to report our findings in a narrative manner as there was insufficient data to carry out a pooled meta-analyses. In studies where several outcomes (e.g asthma, eczema) were studied apart from food allergy or its subtypes, only the results relevant to food allergy and/or its subtypes were included in the final summary of reported associations. In studies where both atopic controls and non-atopic controls were used, only data pertaining to unaffected non-atopic controls were shown.

5.3.4 Results

Characteristics of included studies

A total of 32 articles out of 2088 reviewed met our eligibility criteria (**Figure 5.1**). The characteristics of included studies are summarized in Online Repository Table S4.

Two of these studies were gene-environment interaction studies and these studies reported that the genetic associations were only relevant in the presence of mentioned particular environmental component (250, 251).

We also identified five GWAS with either food or peanut allergy as an outcome. Four of these were carried out in children (252-255) and the other was carried out in a population across the ages of 1 to 93 years (256).

The remaining 25 articles were candidate gene studies, with 14 studies examining food allergy generally, whereas three looked at cow's milk allergy and eight at peanut allergy specifically. Of the 25 candidate gene studies, three of these studies were carried out in a population across ages ranging from 1 to 61 years old, while the remaining 22 studies were in children and young adults under 21 years of age.

Included studies were of varying sample sizes with the smallest study having 30 food allergy Caucasian cases and 35 non-allergic Caucasian controls (257) while the largest study was a GWAS with 2197 European subjects (671 with food allergy, 144 non-allergic non-sensitised controls, and 1,382 European controls of uncertain phenotype) (252). The majority (n=11) of included studies were conducted in only 'Caucasian', 'European' or 'White' populations (254, 256-265), whereas 11 studies were carried out in predominantly Caucasian populations alongside other ethnicities ('Asians', 'Mixed', 'African American') (250-253, 255, 266-271). Four others were carried out in Japanese populations (272-275), one in a Taiwanese population (276), and there were five studies where ethnicity was not mentioned (277-281).

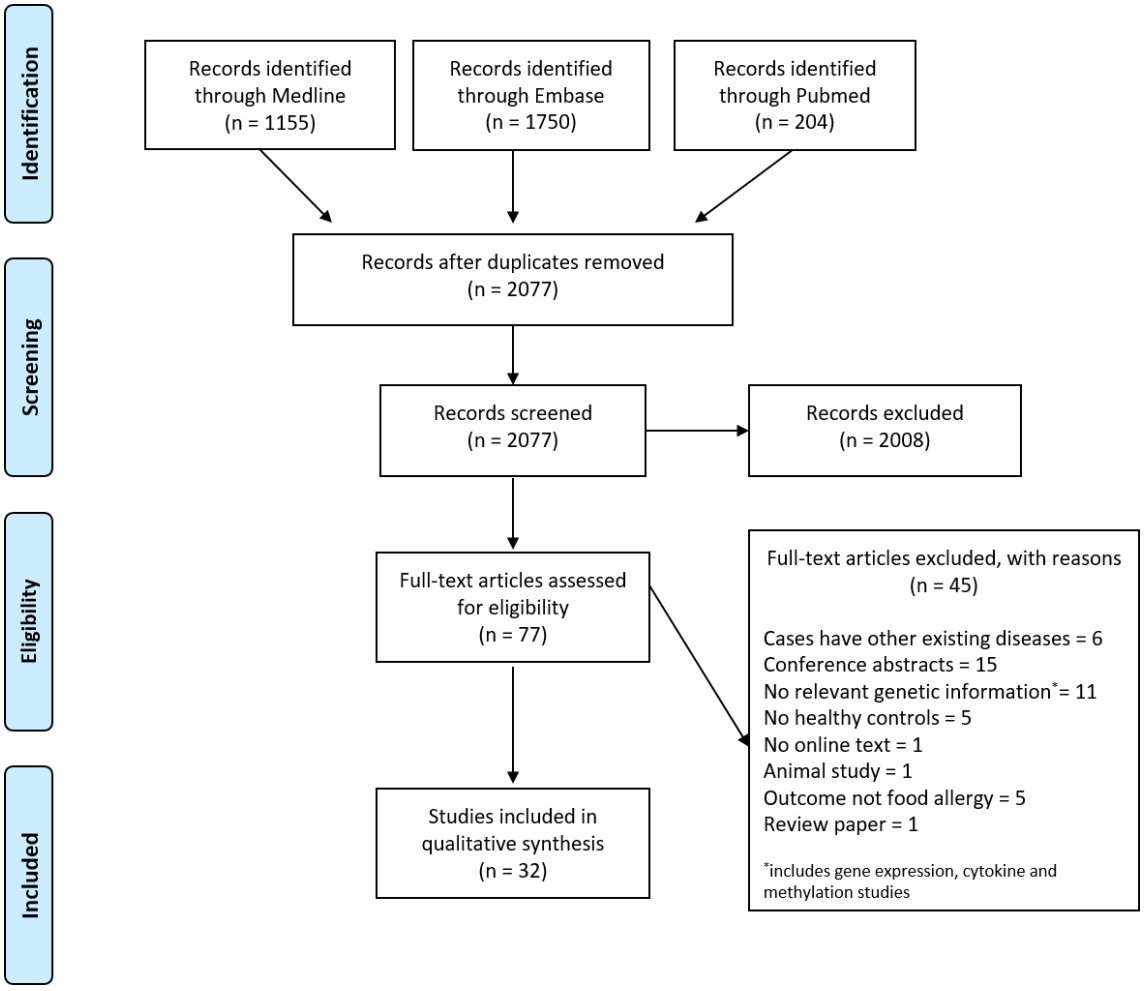

Figure 5.1 Flowchart of literature search process according to PRISMA 2009 flow diagram.

Quality Assessment

A detailed assessment of study quality can be found in the Online Repository Table S3. 17 of the included studies were of low quality, 10 of moderate quality and the remaining five scored highly. Only half of the studies provided information on Hardy-Weinberg equilibrium (HWE) assessment (**Figure 5.2**). In terms of study design, few studies (n=6) included a measure of statistical power as part of their study. 78% of the studies included assessment of population stratification, including those that were not scored on this criteria as they restricted their analyses to one population group (**Figure 5.2**). Several studies carried out meta-analyses with additional cohorts instead of having a replication cohort, but the results were not included in this review. Only 25% of the studies carried out an independent replication cohort to validate their findings.

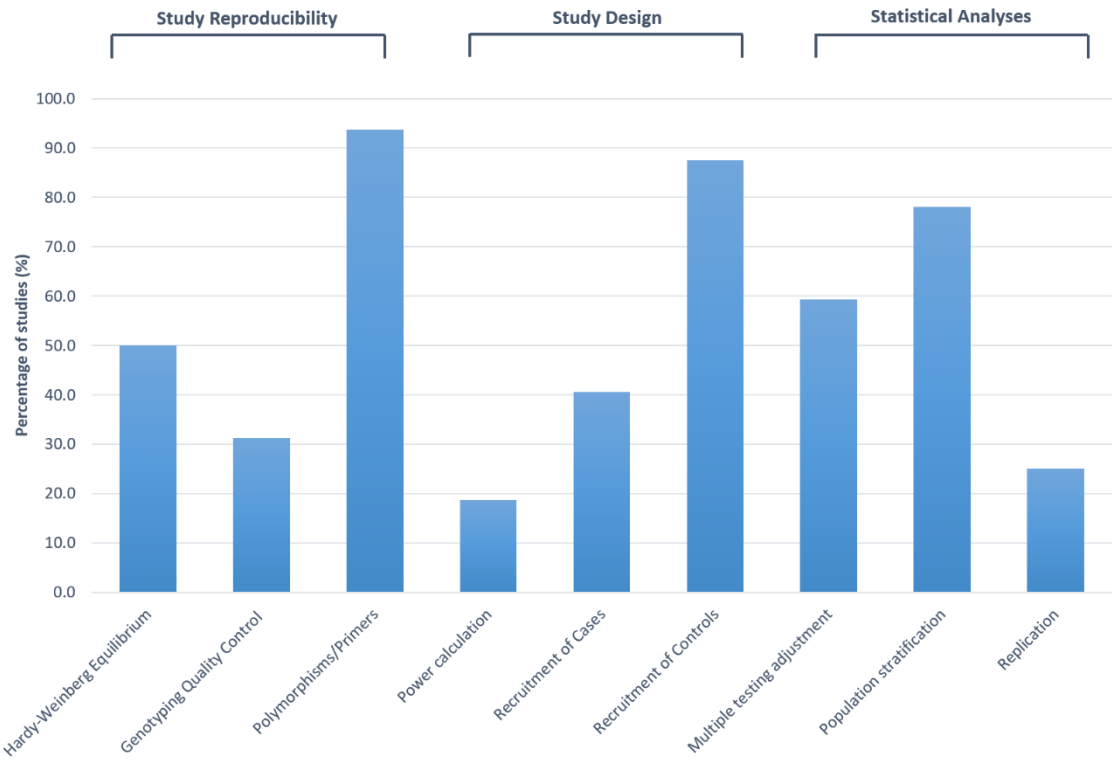

Figure 5.2 Percentage of studies that meet each of the criteria in risk assessment.

Genes investigated in included studies

We identified seven gene regions investigated in more than one study and presented the congruency of their findings here. A summary of these gene regions and the evidence of association with food allergy is shown in **Figure 5.3**. A detailed compilation of genes and SNPs from all eligible studies is provided in Table 5.1.

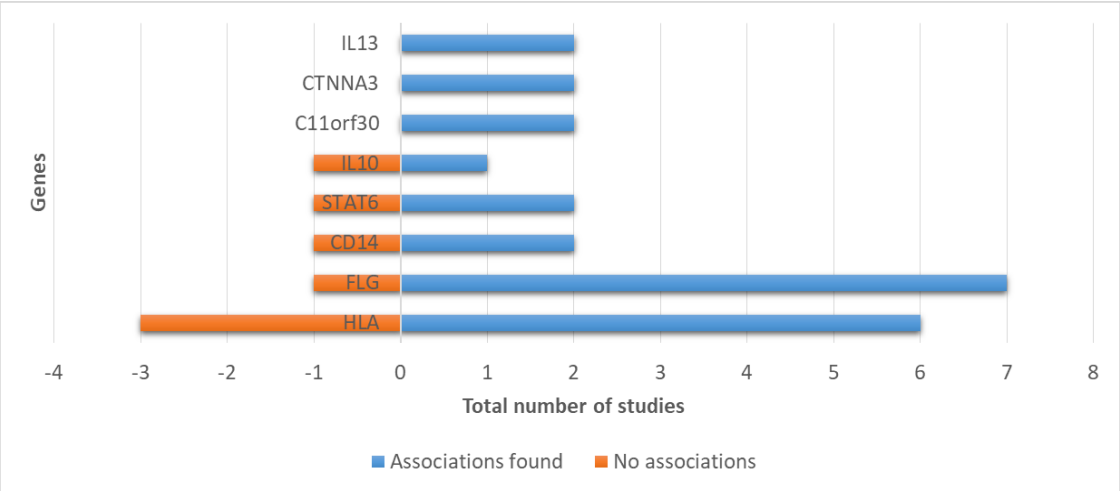

Figure 5.3 Genes/gene regions that were investigated in more than one study and their associations with any type of food allergy. Studies were classified as showing an association if they are associated with any food allergy after multiple testing correction. This included studies that showed suggestive or marginal significant associations. P values used were those determined by each study.

HLA

Human leukocyte antigen (HLA) complex has been one of the most commonly studied gene regions in current food allergy research. This gene has been investigated by nine studies, although with inconsistent findings. Studies investigating *HLA* were widely heterogeneous primarily due to the highly polymorphic nature of the HLA region and the different variant classes. Some studies analysed the classical two or four digit alleles while others analysed the specific HLA protein, amino acid polymorphisms or SNPs within the gene. A study by Li et al however was the only study that investigated candidate genes as well as CNV and CNV regions (CNVR) in a genome-wide dataset (253). In its candidate gene analysis, rare CNVs of duplication in the gene HLA-B at

chr6:31300691-31304663 was detected in two food allergy cases and three control samples.

Associations with SNPs

In the first GWAS of food allergy by Hong et al (n=2694 post-quality control), no polymorphism in the HLA region was found to reach the genome-wide significance level or suggestive threshold in the discovery cohort with the outcome of 'any food allergy' (252) (Table 1). When analysed for specific food allergy such as peanut, egg and milk allergy, two polymorphisms (non-synonymous mutation rs7192 of HLA-DRA and rs9275596 intergenic SNP between HLA-DQB1 and HLA-DQA2) were associated with an increased risk of peanut allergy only, and these findings were replicated in an independent cohort. However, this association was only observed in children of European ancestry and not non-European ancestry. These variants, rs7192 ($r^2 = 0.25$) and rs9275596 ($r^2 = 0.48$) were found to be in linkage disequilibrium with a 3' UTR variant, rs9273440 of HLA-DQB1, which was significantly associated with peanut allergy in another GWAS of food allergy (254).

Association with broad allele groups

Six of the nine studies that investigated *HLA* found associations with broad allele groups. With the exception of Savihlati et al who focused on cow's milk allergy (280), the remaining five studies investigated *HLA* in relation to peanut allergy (255, 262, 263, 269, 270). Savilahti et al did not find any significant associations with cow's milk allergy for HLA class II DR and DQ haplotypes (280). Meanwhile, Howell et al reported an amino acid variant (DRB1*08/12 - tyr16) and two alleles (DRB1*08, DQB1*04) that showed an increased proportion in peanut allergic individuals compared to controls, even after multiple testing correction (262). Two other allele groups, DQB1*02 and DQB1*05 were lower in peanut allergy cases compared to controls (263). Analysis of specific HLA proteins in the same study found a higher frequency of DQB1*06:03P, but a decreased frequency of DQB1*03:02 and DQB1*05:01P in peanut allergy cases compared to controls (263). The letter 'P' added at the end of the allele represent alleles that share the same peptide binding domains (282).

Apart from the Howell et al and Madore et al studies, three other studies reported an association with peanut allergy, but these associations did not survive multiple testing adjustment (255, 269, 270) (Table 1). These studies had smaller sample sizes in comparison to other studies which may have contributed to the lack of association. In Shreffler et al's study carried out in discordant sibling pairs, none of the alleles investigated were associated with peanut allergy in 73 cases (270). However, the DQ7 serotype frequency was higher in sibling controls than those with peanut allergy. In the other study of 84 cases, DRB1*13 and DQB1*06 alleles were higher in cases than controls (269). The last study found an association between reduced risk of peanut allergy and two amino acid variants, which were in linkage disequilibrium in the HLA-DRB1 gene (positions 37 and 71) (255). The association between peanut allergy and the variant at position 71 was initially discovered by Hong et al (252), but it did not remain significant in the replication cohort.

FLG

Similarly, the gene encoding filaggrin (*FLG*) was also commonly investigated with seven studies investigating association of different *FLG* variants with peanut, cow's milk or food allergy (254, 259, 260, 265, 273, 280, 281) and one study investigating the association in the presence of an environmental exposure (gene-environment interaction) (250). Similar to the studies on *HLA*, these studies tend to investigate different combinations of loss-of-function *FLG* mutations, making direct comparisons between the studies challenging. The combination of mutations investigated for each of these studies is shown in Table 1.

Six studies reported a significant association with either food allergy or peanut allergy in the presence/absence of environmental exposure (254, 259, 260, 265, 273, 281). Cases were reported to have a higher proportion of loss-of-function mutations (259, 265) and individuals with loss-of-function mutations (260, 265, 273, 281) or 'T' allele of intron variant, rs12123821 (254) were at least two times more likely to have food or peanut allergy than the control group. However, in a birth cohort study where participants were followed up prospectively for 18 years, an association between food allergy and *FLG* mutations was only observed at 10 and 18 years but not at younger ages (at 1, 2 and 4

years old) (281). It may be that *FLG* mutations are less strongly associated with food allergies that predominate in younger children, such as egg and milk.

The study on cow's milk allergy by Savilahti et al did not find any significant associations with cow's milk allergy for any of their investigated *FLG* polymorphisms (combined del22824, 501-C/T, R2447X, S3247, 3702delG) (280).

One of the identified studies, Brough et al, investigated effect modifications of genetic polymorphisms in *FLG* on the association between peanut allergy and peanut exposure (250). In this study, 9% of all children (N=623) had a loss-of function *FLG* mutations (combined R501X, S3247X, R2447X, 2282del4, 3673delC and/or 3702delG) whereas in peanut allergy cases, 4 out of 20 (20%) carried the loss-of function *FLG* mutations. In the multivariate model, children with one or more *FLG* mutations had a 3.3 times increased odds of peanut allergy with each natural log (ln [log e]) unit increase in house dust peanut exposure. On the other hand, no association between peanut exposure and peanut allergy or peanut sensitisation was observed in children with the wild type *FLG* genotype.

CD14

Three small studies (N <200 subjects in each study) investigated the association of cluster of differentiation 14 (CD14) gene and food allergy (268, 271, 272). These studies all investigated the 5' UTR variant –159 C/T (rs2569190) but obtained conflicting results. Dreskin et al found the C allele to be associated with peanut allergy (268). Conversely, Woo et al (271) found a higher proportion of T alleles in food allergy cases than the controls in both codominant and dominant recessive models, while Campos et al (272) found no evidence of an association between this polymorphism and food allergy. However, it is worth noting that the two latter studies were carried out in different populations – the Woo study predominantly Caucasian with some mixed ethnicity (African American or others not specified) while the Campos study was carried out in a Japanese population.

STAT6

Three studies investigated the associations between polymorphisms within gene encoding signal transducer and activator of transcription 6 (*STAT6*) and nut allergy (258), food allergy (273) or food-related anaphylaxis (275). The G allele of 3' UTR variant 2964G/A

(rs324015) was found at an increased frequency in Caucasian children with nut allergy (258). This same variant, however, was not associated with food-related anaphylaxis in Japanese children (275). The last study on food allergy found an association with a 5' UTR variant in the STAT6 region, rs167769 (273), which was previously associated with eosinophilic esophagitis (283).

IL10

Chen et al (276) and Jacob et al (277) investigated variants at the gene encoding interleukin 10 (*IL10*) in relation to any food and cow's milk allergy, respectively. A common SNP investigated by both studies is the -1082 A/G (rs1800896) variant, a 2 kilo base pair (kb) upstream variant. Jacob et al found that the GG allele for -1082 A/G (rs1800896) was more common in the cow's milk allergy group than the control group (277). Moreover, the *IL10* -3575A, *IL10* -2849A, *IL10* -2763C, *IL10* -1082G and *IL10* -592C haplotype was also higher in cases (10%) than controls (2%). On the other hand, Chen et al did not find an association of any food allergy (milk inclusive) with either the same variant -1082 A/G (rs1800896) or -592 A/C (rs1800872) variant (276).

IL13

Two studies found an association between food allergy and interleukin 13 (*IL13*) intron variant, rs1295686 (267, 273). Both studies observed an increased risk of food allergy among those with the risk allele (A/T). Interestingly, the studies were carried out in different populations, with the Ashley et al (267) study conducted in a Caucasian population using a tag-SNP selection approach, while the Hirota et al (273) study was done in a Japanese population investigating genes previously associated with atopic dermatitis and/or eosinophilic esophagitis.

C11orf30/LRRC32

Hirota et al (273) investigated the association of food allergy with 26 genes previously associated with atopic diseases and eosinophilic esophagitis in GWAS. In this study, a locus within the chromosome 11 open reading frame 30/ leucine-rich repeat-containing protein 32 (*C11orf30)/LRRC32)* region was one of 14 loci found to be associated with

food allergy at the nominal level ($p<0.05$). rs11236809, a 500 base pair downstream variant, was associated with food allergy. In another study by Marenholz et al, an intergenic variant (rs2212434) within the same *C11orf30/LRRC32* region was also associated with food allergy in the discovery cohort and two independent replication cohorts (254). Additionally, Asai et al (256) found an association between peanut allergy and rs7936434, a variant 30kb from *C11orf30*. Collectively, these three studies point towards the association of the region with food or peanut allergy but none investigated the same SNPs for comparison.

Other genes

There were several other studies that investigated genetic associations with food allergy, namely *NLRP3 (274), FcyRIIa (264), IDO(278), NAT2 (261), SPINK5 (266), IL28B (IFNL3) (257), SERPINB (254), TGFb1(277), TLR2* and *TLR4 (279).*

NLRP3 was not found to be associated with food allergy, however, some of the investigated SNPs were found to be associated with food-related anaphylaxis (274).

NAT2, SERPINB and *SPINK5* were reported to be associated with food allergy in a single study each, while the remaining studies of the other genes found no association. Of particular significance is the *SERPINB* gene cluster, a newly identified region associated with challenge-proven food allergy. The association was identified in a GWAS carried out using data from the German Genetics of Food Allergy Study (GOFA) (254). One of the SNPs located in the intron of *SERPINB*, rs12964116, did not remain significant after multiple testing correction in a GOFA replication cohort, but was associated with food allergy when investigated in a second independent replication cohort. Additionally, *SPINK5* variant rs9325071, which has been shown to decrease expression of SPINK5 in the skin, was associated with challenge-proven food allergy in both the discovery and replication cohorts (266).

In Li et al (253), CNVR in *ODZ3, CTNNA3, LUZP2, RBFOX1* and *MACROD2* were found to be associated with food allergy. The *CTNNA3* region was also associated with peanut allergy in another study, where intron variant, rs7475217, was associated with a reduction in the risk of peanut allergy (256).

Apart from these genes, the second gene-environment interaction study investigated polymorphisms of the vitamin D binding protein gene, *GC*, which were found to modify the association between vitamin D levels and food allergy (251). Vitamin D insufficiency (≤ 50 nM/L) at 1 year was associated with food allergy in infants with the GG genotype of rs7041, but not in those with GT or TT genotypes. However, the study did not examine for an association between *GC* and food allergy, independently of vitamin D levels.

Table 5.1 Summary of investigated genes in included studies

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
ABCB11	Hong, 2015 (252)	rs16823014	GWAS	Egg allergy	No ORs given	$4.4x10^{-6}$	N	
ARHGAP24	Asai, 2017 (256)	rs744597	GWAS - Meta analyses	Peanut allergy	0.61 (0.5-0.74)	$3.98x10^{-7}$		
ATP10A	Martino, 2017 (255)	rs17555239	GWAS	Peanut allergy	2.58	$3x10^{-5}$	OR= 0.79, p= 0.131	
BCAS1	Martino, 2017 (255)	rs11700330	GWAS	Peanut allergy	0.23	$3x10^{-6}$	N	
C11orf30/LRRC32	Marenholz, 2017 (254)	rs2212434	GWAS	Food allergy	1.29	3.4×10^{-4}	OR = 1.47, p = 8.2×10^{-5} (Replication 1) p=1.4 × 10−4 (Replication 2)	N
C11orf30/LRRC32	Hirota, 2017 (273)	rs11236809	Candidate gene	Food allergy	1.34 (1.14-1.59)	0.00056	OR= 1.33 (1.08-1.63), p=0.0096, Pcombined=0.000014	N
C11orf30/ LOC101928813	Asai, 2017 (256)	rs7936434	GWAS - Meta analyses	Peanut allergy	1.58 (1.32-1.9)	$5.17x10^{-7}$		

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
CCDC80	Hirota, 2017 (273)	rs12634229	Candidate gene	Food allergy	1.26 (1.08-1.46)	0.0039	OR=1.24 (1.02-1.52), p=0.030, Pcombined=0.00028	
CD14	Campos, 2007 (272)	CD14 -159 (rs2569190)	Candidate gene	Food allergy	No ORs given, only frequencies	0.8		✓(268, 271)
CD14	Campos, 2007 (272)	CD14 -550 (rs5744455)	Candidate gene	Food allergy	No ORs given, only frequencies	0.8		N
CD14	Dreskin, 2011 (268)	rs2569193	Candidate gene	Peanut allergy	1.33 (0.53–3.34)	0.54		
CD14	Dreskin, 2011 (268)	rs2569190	Candidate gene	Peanut allergy	1.97 (1.02–3.79)	0.04		✓(271) X (272)
CD14	Woo, 2003 (271)	–159 C/T (rs2569190)	Candidate gene	Food allergy	1.7 (1.1-2.8)	0.03		✓(268) X (272)
CHCHD3 /EXOC4	Asai, 2017 (256)	rs78048444	GWAS	Peanut allergy	0.22 (0.12-0.39)	$5.44x10^{-7}$		
CLEC16A/DEXI	Hirota, 2017 (273)	rs2041733	Candidate gene	Food allergy	1.15(0.99-1.35)	0.074	OR=1.18 (0.96-1.45), p=0.12, Pcombined=0.019	
COG7	Hong, 2015 (252)	rs250585	GWAS	Egg allergy	No ORs given	$3.8x10^{-6}$	N	
CTNNA3	Asai, 2017 (256)	rs7475217	GWAS	Peanut allergy	1.64 (1.35-1.98)	$3.58x10^{-7}$		N

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
CTNNA3	Li, 2015 (253)	chr10:68282970-68284017 chr10:68383827-68407077	GWAS	Food allergy		0.0184	p=0.0206	N
EMCN	Hong, 2015 (252)	rs1318710	GWAS	Food allergy	No ORs given	$2.6x10^{-6}$	N	
FAM117A	Hong, 2015 (252)	rs9898058	GWAS	Milk allergy	No ORs given	$1.1 x10^{-6}$	N	
FcyRIIa	Pawlik, 2004 (264)	Not given	Candidate gene	Food allergy	No ORs given, only frequencies	None significant (p-values not given)		
FLG	Brown, 2011 (260)	Combined null genotype R501X and 2282del4	Candidate gene	Peanut allergy	English: 3.2 (1.4-7.2) English, Dutch, Irish: 5.3 (2.8-10.2)	0.0251 $3.0x 10^{-6}$	N	N
FLG	Brown, 2011 (260)	Combined null genotype R501X, 2282del4, R2447X, and S3247X	Candidate gene	Peanut allergy	Dutch: 3.5 (1.1-11.4) Irish: 3.3 (1.0-11.7)	0.0335 0.0640	OR=1.9 (1.4-2.6), P= $5.4 x 10^{-5}$	N

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
FLG	Hirota, 2017 (273)	rs6696556	Candidate gene	Food allergy	1.05 (0.84-1.31)	0.68	OR=1.15 (0.86-1.54), P=0.37, Pcombined=0.39	N
FLG	Hirota, 2017 (273)	p.S2889*	Candidate gene	Food allergy	2.32 (1.37-3.98)	0.001	Replication: OR=2.41 (1.27-4.49), p=0.0049 Combined: OR=2.36 (1.58-3.52), P=0.000015	N
FLG	Hirota, 2017 (273)	6 FLG null variants, c.3321delA, p.Q1701*, p.S2554*, p.S2889*, p.S3296*, and p.K4022*	Candidate gene	Food allergy	1.42 (1.04-1.92)	0.024	Replication: OR=2.04 (1.38-3.01), p=0.00035 Combined: OR=1.63 (1.28-2.07), P= 0.000055	N
FLG	Savilahti, 2010 (280)	5 filaggrin null mutations (del22824, 501-C/T, R2447X, S3247, 3702delG)	Candidate gene	Cow's milk allergy	No ORs given, only frequencies	None significant (p>0.003)		✓ (265, 281)
FLG	Venkataraman, 2014 (281)	5 polymorphisms (R501X, 2282del4, S3247X,	Candidate gene	Food allergy	10 years: 2.9 (1.2-7.0) 18 years: 2.5 (1.2- 5.3)	0.022 0.032		✓(265) X (280)

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
		3702delG, and R2447X)						
FLG	Brough, 2014 (250)*	Combined null mutations R501X, S3247X, R2447X, 2282del4, 3673delC and 3702delG	G x E	Peanut allergy	Univariate: 2.70 (0.9-8.0) Multivariate: 3.2 (1.1-9.8)	0.07 0.04		N
FLG	Tan, 2012 (265)	R501X, 2282del4, R2447X, S3247X, and 3702delG	Candidate gene	Food allergy	3.2 (1.2-8.5)	0.016 (0.055 after adjusting for eczema)		✓(281) X (280)
FLG	Asai, 2013 (259)	Combined rs61816761, rs41370446, rs138726443, rs150597413	Candidate gene	Peanut allergy	1.96 (1.49-2.58)	5.12×10^{-7}		N
FLG-AS1	Marenholz, 2017 (254)	rs12123821	GWAS	Food allergy	2.55	8.4×10^{-10}	OR=2.86, p= 6.1×10^{-7} (Replication 1)	N
FXR1	Martino, 2017 (255)	rs6763069	GWAS	Peanut allergy	0.38	2×10^{-5}	N	
GC	Koplin, 2016 (251)*	Combined rs7041 and rs4588	G x E	Food Allergy	6.0 (0.9-38.9)	Pinteraction= 0.014		

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
GLB1	Hirota, 2017 (273)	rs6780220	Candidate gene	Food allergy	1.40 (1.21-1.62)	0.0000082	OR=1.20 (0.99-1.45), p=0.064 Pcombined=0.0000025	
HLA	Martino, 2017 (255)	Amino acid polymorphisms at position 37	GWAS	Peanut allergy	0.3 (0.16-0.55)	$9.8x10^{-5}$	N	N
HLA	Martino, 2017 (255)	Amino acid polymorphisms at position 71	GWAS	Peanut allergy	0.34 (0.19-0.59)	$1.5x10^{-4}$	N	✓(252)
HLA	Savilahti, 2010 (280)	HLA class II haplotypes (DQB1, DRB1, DQA1)	Candidate gene	Cow's milk allergy	No ORs given, only frequencies	None significant (p>0.003)		N
HLA	Howell, 1998 (262)	DRB1*08	Candidate gene	Peanut allergy	No ORs given, only frequencies	0.0021 (Pcorrected=0.027)		N
HLA	Howell, 1998 (262)	DBR1*08/12 (tyr16)	Candidate gene	Peanut allergy	No ORs given, only frequencies	0.0023 (Pcorrected=0.029)		N
HLA	Howell, 1998 (262)	DQB1*04	Candidate gene	Peanut allergy	No ORs given, only frequencies	0.00042 (Pcorrected=0.0029)		N
HLA	Shreffler, 2006 (270)	DR11	Candidate gene	Peanut allergy	No ORs given, only frequencies	0.07 (Pcorrected=1.3)		N
HLA	Shreffler, 2006 (270)	DQ7	Candidate gene	Peanut allergy	No ORs given, only frequencies	0.04 (Pcorrected=0.3)		N

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
HLA	Shreffler, 2006 (270)	6 DQ serotypes (DQ2, DQ4, DQ5, DQ6, DQ8, and DQ9) and 17 DR allele groups (DR1, DR4, DR7, DR8, DR9, DR10, DR12, DR13, DR14, DR15, DR16, DR17, DR18, DR51, DR52, DR53, and DR103)	Candidate gene	Peanut allergy	No ORs given, only frequencies	None significant (P/Pcorrected>0.05)		N
HLA- DQB1	Madore, 2013 (263)	DQB1*06:03P	Candidate gene	Peanut allergy	2.59 (1.56–4.44)	1.6x 10-04, Pc=1.9x10^{-3}		N
HLA- DQB1	Madore, 2013 (263)	DQB1*02	Candidate gene	Peanut allergy	0.12 (0.07–0.21)	1.1 x 10-16, Pcorrected=1.3x10^{-15}		N
HLA- DQB1	Madore, 2013 (263)	DQB1*03:02P	Candidate gene	Peanut allergy	0.52 (0.34–0.79)	2.2 x10-03, Pcorrected=2.6x10^{-2}		N

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
HLA- DQB1	Madore, 2013 (263)	DQB1*05	Candidate gene	Peanut allergy	0.21 (0.08–0.50)	2.5 x10-04, Pcorrected=3.0x10^{-3}		N
HLA- DQB1	Madore, 2013 (263)	DQB1*05:01P	Candidate gene	Peanut allergy	0.25 (0.13–0.47)	7.7x 10-06, Pcorrected=9.3x10^{-5}		N
HLA-A, B, DRB1, DQB1	Hand, 2004 (269)	B*07, DRB1*11	Candidate gene	Nut allergy	No ORs given, only frequencies	None significant (P>0.05)		N
HLA-A, B, DRB1, DQB1	Hand, 2004 (269)	DRB1*13	Candidate gene	Nut allergy	No ORs given, only frequencies	<0.05 (Pcorrected=0.82)		N
HLA-A, B, DRB1, DQB1	Hand, 2004 (269)	DQB1*06	Candidate gene	Nut allergy	No ORs given, only frequencies	<0.01 (Pcorrected=0.37)		N
HLA-B	Li, 2015 (253)	chr6:31300691-31304663	GWAS	Food allergy	Not given	Not given	p = 0.026 , Pcombined= 0.063	N
HLA-DQB1	Marenholz, 2017 (254)	rs9273440	GWAS	Peanut allergy	0.66	6.6×10^{-7}	OR=0.45, p=3.8×10^{-6} (Replication 1)	N
HLA-DQB1 and HLA-DQA2	Hong, 2015 (252)	rs9275596	GWAS	Peanut allergy	European: 1.7 (1.4-2.1) Non-European: 1.2 (0.8-1.8)	6.8x10^{-10} 0.327	OR=1.7 (1.1-2.6), p=0.022 OR=0.6 (0.2-1.3), p=0.176	N

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
HLA-DRA	Hong, 2015 (252)	rs7192	GWAS	Peanut allergy	European: 1.7 (1.4-2.1) Non-European: 1.2 (0.8-1.8)	$5.5x10^{-8}$ 0.198	OR=1.8 (1.2-2.7), p=0.005 1.4 (0.7-3.1), p=0.375	N
HMGA2|LLPH	Hong, 2015 (252)	rs10878354	GWAS	Peanut allergy	Not given	$5.1x10^{-6}$	N	
IDO1 and IDO2	Buyuktiryaki, 2016 (278)	10 SNPs: rs3808606, rs3824259, rs10089084, rs6991530, rs10504013 rs11992749, rs10109853, rs4503083, rs2955903, rs7820268	Candidate gene	Food allergy	No ORs given, only frequencies	None significant (P values >0.05)		
IER5L	Martino, 2017 (255)	rs4240433	GWAS	Peanut allergy	3.61	$7x10^{-6}$	OR=0.83, 0.316	
IL10	Abe Jacob, 2013 (277)	IL10 -1082	Candidate gene	Cow's milk allergy	No ORs given, only frequencies	0.027 (Pcorrected= 0.054)		X (276)

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
IL10	Chen, 2012 (276)	-1082 A/G (rs1800896) and -592 A/C (rs1800872)	Candidate gene	Food allergy	No ORs given, only frequencies	0.994 0.770		✓(277)
IL13	Ashley, 2017 (267)	rs1295686	Candidate gene	Food allergy	1.75 (1.20-2.53)	0.003	OR=1.37 (1.03-1.82), p=0.03	
KIF3A/IL13	Hirota, 2017 (273)	rs1295686	Candidate gene	Food allergy	1.44 (1.23-1.68)	0.0000031	OR=1.34 (1.10-1.64), p=0.0038, Pcombined=0.000000067	
IL2/IL21	Hirota, 2017 (273)	rs17389644	Candidate gene	Food allergy	1.14(0.90-1.44)	0.28	OR=1.49 (1.13-1.97), p=0.0049, Pcombined=0.0096	
IL26	Martino, 2017 (255)	rs7300806	GWAS	Peanut allergy	0.28	$1x10^{-5}$	OR=0.82, p= 0.319	
IL28B	Gaudieri, 2012 (257)	rs12979860	Candidate gene	Food allergy	Cohort 1: 4.56 (1.7–12.6) Cohort 2: 3.0 (1.8–5.2)	0.004 0.04		
IL4/KIF3A	Marenholz, 2017 (254)	rs11949166	GWAS	Food allergy	0.6	1.2×10^{-13}	OR=0.69, p=3.0×10^{-5} (Replication 1)	
IMPAD1|LOC286177	Hong, 2015 (252)	rs7833294	GWAS	Milk allergy	No ORs given	$7.3x10^{-6}$	N	

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
ITIH5L	Hong, 2015 (252)	rs5961136	GWAS	Egg allergy	No ORs given	$2.4x10^{-6}$	N	
LINGO2	Martino, 2017 (255)	rs10812871	GWAS	Peanut allergy	0.38	$4x10^{-5}$	OR=0.68 p=0.014*	
LMX1A	Martino, 2017 (255)	rs6686894	GWAS	Peanut allergy	0.06	$4x10^{-7}$	OR=1.29, p=0.280	
LOC100129104|ZFAT	Hong, 2015 (252)	rs4584173	GWAS	Peanut allergy	No ORs given	$3.6x10^{-6}$	N	
LOC100289292|ETAA1	Hong, 2015 (252)	rs17032597	GWAS	Milk allergy	No ORs given	$1.6x10^{-6}$	N	
LOC100289677|TP53TG1	Hong, 2015 (252)	rs6942407	GWAS	Food allergy	No ORs given	$8.2x10^{-6}$	N	
LOC645314|SLC39A10	Hong, 2015 (252)	rs777717	GWAS	Food allergy	No ORs given	$4.7x10^{-6}$	N	
LOC729993|ERCC4	Hong, 2015 (252)	rs6498482	GWAS	Egg allergy	No ORs given	$4.8x10^{-6}$	N	
LSP1	Hong, 2015 (252)	rs78405116	GWAS	Milk allergy	No ORs given	$1.7x10^{-6}$	N	
LUZP2	Li, 2015 (253)	chr11:247789612 4783183 chr11:24412621-24551109	GWAS	Food allergy	No ORs given, only frequencies	0.0226	p=0.0153	

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
MACROD2	Li, 2015 (253)	chr20:151041931 5126507 chr20:14713890-14727386	GWAS	Food allergy	No ORs given, only frequencies	3.37 x10^{-3}	p=1.41 x10^{-3}	
MDN1	Martino, 2017 (255)	rs9362681	GWAS	Peanut allergy	2.83	1x10^{-5}	OR=1.43, p=0.037*	
NAT2	Gawronska-Szklarz, 2001 (261)	NAT2*4 (fast acetylator), NAT2*5, NAT2*6, and NAT2*7 (slow acetylators)	Candidate gene	Food allergy	No ORs given, only frequencies	P <0.001		
NAV2	Martino, 2017 (255)	rs2439871	GWAS	Peanut allergy	0.38	1x10^{-5}	OR=0.94, p=0.723	
NLRP3	Hitomi, 2009 (274)	rs12079994	Candidate gene	Food-induced anaphylaxis	1.81 (1.09–2.99)	0.021		
NLRP3	Hitomi, 2009 (274)	rs4925650	Candidate gene	Food-induced anaphylaxis	1.77 (1.26–2.49)	0.00091		
NLRP3	Hitomi, 2009 (274)	rs3806265	Candidate gene	Food-induced anaphylaxis	1.71 (1.20–2.43)	0.0029		
NLRP3	Hitomi, 2009 (274)	rs4612666	Candidate gene	Food-induced anaphylaxis	1.81 (1.27–2.56)	0.00086		

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
NLRP3	Hitomi, 2009 (274)	rs10925026	Candidate gene	Food-induced anaphylaxis	1.53 (1.09–2.16)	0.013		
NLRP3	Hitomi, 2009 (274)	rs10754558	Candidate gene	Food-induced anaphylaxis	1.80 (1.28–2.54)	0.00068		
NLRP3	Hitomi, 2009 (274)	rs10733112	Candidate gene	Food-induced anaphylaxis	1.71 (1.21–2.40)	0.0021		
NLRP3	Hitomi, 2009 (274)	rs2027432, rs4925648, rs12048215, rs10754555, rs10925019, rs4925654, rs12565738, rs4378247	Candidate gene	Food allergy	No ORs given	None significant (p>0.05)		
ODZ3	Li, 2015 (253)	chr4:183271349183291465 chr4:183559306-183565618	GWAS	Food allergy	No ORs given, only frequencies	0.0116	0.018	
OR10A3/NLRP10	Hirota, 2017 (273)	rs878860	Candidate gene	Food allergy	1.10 (0.95-1.27)	0.21	OR=1.29 (1.07-1.57), p=0.01, Pcombined=0.01	

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
OVOL1	Hirota, 2017 (273)	rs593982	Candidate gene	Food allergy	1.23 (1.06-1.42)	0.0049	OR=1.04 (0.86-1.26), p=0.72, Pcombined=0.016	
PAFAH1B1	Martino, 2017 (255)	rs8077351	GWAS	Peanut allergy	0.05	$3x10^{-5}$	OR=1.07, p=0.820	
PAX2	Martino, 2017 (255)	rs6584390	GWAS	Peanut allergy	3.56	$4x10^{-5}$	OR=1.03, p=0.864	
PLAGL1	Martino, 2017 (255)	rs6928827	GWAS	Peanut allergy	13.98	$1x10^{-7}$	OR=0.77, p=0.292	
PTPN22	Savilahti, 2010 (280)	R620W (rs2476601)	Candidate gene	Cow's milk allergy	No ORs given, only frequencies	None significant (p-value > 0.003)		
PYROXD1	Martino, 2017 (255)	rs7131777	GWAS	Peanut allergy	2.55	$4x10^{-5}$	N	
RBFOX1	Li, 2015 (253)	chr16:71266297196046 chr16:6763216-6801846	GWAS	Food allergy	No ORs given, only frequencies	$4.72x10^{-3}$	0.9989	
RGS21	Martino, 2017 (255)	rs12142904	GWAS	Peanut allergy	3.51	$5x10^{-6}$	OR=1.02, p=0.905	
RHOBTB1	Hong, 2015 (252)	rs10994607	GWAS	Food allergy	No ORs given	7.1x10-6	N	

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
RHOBTB1|TMEM26	Hong, 2015 (252)	rs10994613	GWAS	Milk allergy	No ORs given	4.8x10-6	N	
RIMS2	Martino, 2017 (255)	rs16870788	GWAS	Peanut allergy	3.58	$3x10^{-5}$	OR=0.93, p=0.734	
RNF130	Martino, 2017 (255)	rs864481	GWAS	Peanut allergy	2.91	$5x10^{-5}$	OR=1.09, p=0.681	
SALL3	Martino, 2017 (255)	rs73971133	GWAS	Peanut allergy	0.07	$3x10^{-5}$	OR=0.87, p=0.723	
SERPINB7	Marenholz, 2017 (254)	rs12964116	GWAS	Food allergy	1.9	5.7×10^{-6}	OR=1.69, $p=9.4 \times 10^{-3}$ (Replication 1) p=0.010 (Replication 2)	
SERPINB7/B2	Marenholz, 2017 (254)	rs1243064	GWAS	Hen's egg allergy	1.65	1.6×10^{-7}	OR=1.21, p=0.028 (Replication 1) p=0.15 (Replication 2)	
SGCD	Hong, 2015 (252)	rs7717393	GWAS	Egg allergy	No ORs given	$1.4x10^{-6}$	N	
SKAP1	Asai, 2017 (18)	rs16955960	GWAS	Peanut allergy	2.06 (1.54-2.75)	$1.01x10^{-6}$		
SLC2A9	Martino, 2017 (255)	rs10018666	GWAS	Peanut allergy	5.9	4×10^{-8}	OR=1.18, p=0.360	
SORBS2	Martino, 2017 (255)	rs57144668	GWAS	Peanut allergy	0.37	$3x10^{-5}$	OR=1.50, p=0.014*	

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
SPINK5	Ashley, 2017 (266)	77 tag-SNPs within a region of ~263 kb capturing 387 alleles with LD of r2≥.8	Candidate gene	Food allergy	2.95 (1.49-5.83)	0.001	OR=1.58 (1.13-2.20), p=0.007	
SSBP3|ACOT11	Hong, 2015 (252)	rs12121623	GWAS	Food allergy	No ORs given	$3.1x10^{-7}$	N	
STAT6	Tamura, 2003 (275)	G2964A (rs324015)	Candidate gene	Food-related anaphylaxis	No ORs given, only frequencies	0.4974		✓(258)
STAT6	Amoli, 2002 (258)	2964 G/A 3'UTR (rs324015)	Candidate gene	Nut allergy	2.9 (1.7– 4.9)	< 0.0001		X (275)
STAT6	Hirota, 2017 (273)	rs167769	Candidate gene	Food allergy	1.26 (1.06-1.50)	0.0082	OR=1.24 (0.99-1.56), p=0.06, Pcombined=0.0014	N
STXBP6|NOVA1	Hong, 2015 (252)	rs862942	GWAS	Peanut allergy	No ORs given	$3.0x10^{-6}$	N	
SV2C	Martino, 2017 (255)	rs10474468	GWAS	Peanut allergy	0.37	$5x10^{-5}$	OR=0.84, p=0.261	
TES	Martino, 2017 (255)	rs73220497	GWAS	Peanut allergy	0.06	$3x10^{-5}$	OR=1.04, p=0.891	
TGFb1	Abe Jacob, 2013 (277)	TGFb1 -509C/T	Candidate gene	Cow's milk allergy	No ORs given, only frequencies	0.6419		

Genes of interest	Author	SNPs/CNVs/ alleles	Study type	Outcome	OR (95% CI)	P value[a]	Within-study replication[a b c]	Cross-study replication[d e f]
TLR2	Galli, 2010 (279)	R753Q (rs5743708)	Candidate gene	Cow's milk and allergy	No ORs given, only frequencies	None significant (P values >0.05)		
TLR4	Galli, 2010 (279)	D299G (rs4986790)	Candidate gene	Cow's milk and allergy	No ORs given, only frequencies	None significant (P values >0.05)		
TMEM232/SLC25A46	Hirota, 2017 (273)	rs9326801	Candidate gene	Food allergy	1.33(1.09-1.61)	0.0037	OR= 0.98 (0.75-1.27), p=0.87, Pcombined=0.031	
TNFRSF6B/ZGPAT	Hirota, 2017 (273)	rs6010620	Candidate gene	Food allergy	1.11(0.95-1.29)	0.19	OR=1.19 (0.98-1.46), p=0.082, Pcombined=0.039	
TSLP/WDR36	Hirota, 2017 (273)	rs3806932	Candidate gene	Food allergy	1.19(1.02-1.40)	0.032	OR=1.15 (0.94-1.42), p=0.19, Pcombined=0.012	
ZNF365	Hirota, 2017 (273)	rs10995251	Candidate gene	Food allergy	1.32(1.14-1.53)	0.00017	OR=1.15 (0.95-1.39), p=0.18, Pcombined=0.00013	
ZNF652	Hirota, 2017 (273)	rs16948048	Candidate gene	Food allergy	1.20(0.97-1.47)	0.093	OR=1.41 (1.08-1.82), p=0.0096 Pcombined=0.0039	

CNVs: Copy number variations; GWAS: Genome-wide association study; GxE: Gene-environment interaction studies; OR: odds ratios; SNPs: single nucleotide polymorphisms

[a] Pcombined refers to p-values obtained from the combination of discovery and replication cohort, as given in the respective studies. Pcorrected refers to p-vales after multiple testing correction

[b] Rows shaded grey indicate study did not include a replication cohort.

[c] N to denote studies have replication cohort but SNP/allele was not investigated in replication cohort.

[d] Rows shaded grey diagonally indicate no other studies investigated same gene.

[e] N where there are other studies that investigated the same gene, but investigated SNP/allele differ among studies.

[f] ✓indicate findings are associated with food allergy in cited study. X indicate findings are not associated with food allergy in cited study.

5.3.5 Discussion

This is the first review to systematically collate genetic association studies of food allergy. Overall, studies were of varied quality and reproducibility of findings for the same SNPs were minimal. This is not particularly surprising given genetic association studies in food allergy are still emerging. While a number of discovery studies did not include a replication phase, it is promising to notice that more recent studies are recognising the importance of replication in order to minimise publication of false-positive findings. With the exception of two studies published in 2016 (251, 278), the remaining eight studies published within the past three years all included a replication analysis. Most studies also included an appropriate adjustment for population heterogeneity in the form of a statistical adjustment, an exclusion of mixed/other ethnicities in their statistical analysis, inclusion of ancestry informative markers as genetically inferred ancestry or was mentioned as a limitation of their study. However, several studies failed to address the need for any population adjustment. Assessment of population stratification is essential in genetic studies since any allelic or genotypic frequencies observed may be correlated with ethnicity and not the disease outcome. Apart from population stratification, multiple testing adjustment is also crucial since absence of multiple correction may lead to false positive associations with food allergy. However, 13 of the included studies did not adequately address this criteria.

In this review, we have included studies that have used an OFC as a diagnostic measure for defining food allergy as well as studies using measures of IgE sensitisation in conjunction with history of reaction. Out of the 32 included studies, 11 studies defined food allergy based on history of reactions and SPT, 9 used OFC and the remaining 12 studies used a combination of classifications – an OFC where possible/available and where unavailable, a history of reaction was used instead. Evidently, there is still a paucity of studies using OFC as a definition for food allergy. Use of SPT and history of reaction alone is likely to increase the chances of misclassification of food allergy cases.

Despite these limitations, reproducible associations with food allergy were found for a limited number of genes. The most reproducible association with food allergy is for the *FLG* loss-of-function mutations, which was independently reported in eight studies. *FLG*

encodes for an intermediate filament-associated protein that aggregates keratin intermediate filaments in mammalian epidermis which are important in water retention (284). A loss-of-function mutation in *FLG* would thus potentially increase skin permeability and enhance allergen penetration through the skin (285, 286). This mechanism has been demonstrated in several mouse model studies (287-289). *FLG* variants have also been shown to be associated with eczema and other allergic diseases (290). While there have been several studies investigating *FLG* association with food allergy, we were unable to perform a meta-analysis since only two studies investigated the same set of FLG polymorphisms. Studies of this gene often combine multiple loss-of-function mutations for analysis of association with disease. The combination of loss-of-function mutations investigated differs between studies, often based on the ethnicity of study participants. Nonetheless, currently available data overall support a genuine association between food allergy and *FLG*.

The next most reproducible associations were found between variants at HLA genes DQB1 and DRB1, and peanut allergy phenotypes. The HLA-DR and -DQ molecules are expressed in several cells with antigen presenting capability such as B cells, macrophages and monocytes which are known to play a critical role in the development of allergy. One of the key steps to antigen-specific immune responses is antigen presentation by HLA molecules. As these HLA molecules have specific molecular polymorphisms confined to its peptide binding groove, these polymorphisms may alter the binding affinity of antigen presenting cells for specific peanut peptides (252). In particular, the polymorphic amino acid residue 71 along with position 13, 70 and 74, have been shown to affect the binding specificity of pocket 4, therefore influencing the presentation and interaction of peanut antigens (252, 291). Two SNPs in this region which were associated with peanut allergy, rs7192 and rs9275596, were additionally found to affect DNA methylation and thereby expression levels of HLA-DRB1 and HLA-DQB1 genes (252). The results of this review appear to show a distinction in genetic association based on the type of food. For instance, it is likely that HLA plays a causal role in food allergy, with high specificity to peanut allergy.

A recently identified gene, *C11orf30/LRRC32* has shown promising results for an association with food allergy. The C11orf30/LRRC32 region has previously been associated

with eczema (292-294), asthma (295, 296), serum IgE levels (297) and eosinophilic esophagitis (283). The *C11orf30* encodes the EMSY protein which is responsible for binding of BRCA2 cancer susceptibility gene (298). Given its role in inflammatory diseases, *C11orf30* may play a role in epithelial barrier and differentiation (299). The neighbouring gene, *LRRC32,* is a surface biomarker expressed on regulatory T cells (300) shown to be important in immune tolerance (301). One of the investigated SNPs in this region, rs2212434, was associated with food allergy (254) and an association with eczema was previously identified in a large meta-GWAS on eczema (302). Another SNP in the region was also found to increase the risk of atopic march (rs2155219, 17 kb away from rs2212434) (43), further supporting the role of this region in allergic disease.

Collectively, the involvement of several genes in the mechanism of food allergy points towards the complex and multifactorial nature of food allergy. Like other allergic diseases, the genetic architecture of food allergy appears to involve several relatively common genetic variants of low penetrance and variable expressivity, although the role for rare deleterious mutations has not yet been explored. Some of the genes with evidence for association with food allergy have also been shown to be associated with other allergic diseases such as eczema, asthma and allergic rhinitis. Identifying genes uniquely associated with food allergy is therefore challenging. Some genetic variants may increase overall susceptibility to atopy, such as those in *FCER1A, STAT6, IL13* (303, 304) which are associated with total serum IgE. While these variants can manifest as a number of allergic diseases as well as symptomatic and asymptomatic sensitisation to foods and aeroallergens, others such as those in *HLA* may be specifically associated with reactions to a particular food such as peanut. As such, it is important for future studies to clarify whether the intention is to focus on genetic risk factors specific to food allergy, including specific food allergies such as peanut allergy, or to investigate shared markers for allergic diseases.

Limitations of this systematic review

We restricted our systematic review to paediatric studies since the prevalence of food allergy is known to be the greatest in children compared to adults and the quality of case phenotyping at the population level is higher. We also did not include results of studies that have carried out computer mapping or pathway analyses to find causal food allergy

genes. These studies may provide greater insight into other potentially relevant genes that have not been examined in genetic association studies and may be worth pursuing, but is beyond the scope of the review. Several papers (305-307) that were often quoted in narrative reviews as relevant to food allergy genetic associations were excluded from our systematic review. These studies were excluded primarily because they did not include a healthy control group in their study and/or only investigated genetic associations with regard to severity of food allergy and not the absence/presence of food allergy. We were also unable to carry out meta-analysis on the collated data due to the small number of studies of each locus and the fact that studies investigated different polymorphisms at these loci.

5.3.6 Conclusion

To date there is relatively strong evidence that food allergy is associated with genetic variants at *FLG, HLA, IL13,* as well as some evidence for other variants (*SPINK5, SERPINB, C11orf30*) that warrant further investigation. Although several studies reported promising data to support associations of genetic variants with food allergy, they were compromised by issues of inadequate sample size, absence of multiple testing correction and population stratification. Future investigations would benefit from having larger numbers to improve power and include replication cohorts to validate findings. Further functional research is also necessary to unravel the mechanisms of action of identified novel gene variants responsible for the observed association.

Chapter 6 Selecting candidate genes for genotyping

6.1 Introduction

In the current age of globally mobile societies, studies on health disparities related to immigrant populations are becoming increasingly relevant. Migration has been associated with curious health disparities in a range of disease settings after arrival at the new country (308). We have previously shown that the prevalence of peanut allergy in East Asian infants born in Melbourne (first generation migrants), is above and beyond that of Australian children (49). In this study, differences in parenting practises and household exposures in the first year of life explained less than 18% of the difference in food allergy prevalence and is therefore inconsistent with the idea that purely environmental factors might explain this health disparity. A key paradox was that self-reported allergies in parents of these children were low, arguing against selection bias. Further, available prevalence data suggests that food allergies are substantially less common in Asian infants living in Asia (7, 309). Shared exposures such as adopting Western diets or Western infant feeding practices would confer the same level of risk on these children as experienced by the native population (Australians). Clearly, this is not observed within the HealthNuts cohort, suggesting more complex gene-environment interactions may explain the disparity in food allergy rates in Asian migrants living in Australia. One difficulty, however, in interpreting migrant studies is the relative contribution of genetic versus environmental effects on health disparities, and potential confounding due to selection bias and differences in health-seeking behaviours between migrant versus native populations living in the same locality. These issues pose major challenges for observational studies, limiting current knowledge on the mechanisms and pathways in the development of altered risk of food allergies in migrants.

A plausible hypothesis for the greater risk of food allergy observed in East Asian infants born in Melbourne, dates to the period when ancestral *Homo sapiens* are known to have emigrated from East Africa. In such circumstances, ancestral *Homo sapiens* evolved an immune system selected for living in a tropical environment where infectious and tropical diseases brought about by helminths and parasites thrive. The historical parasite endemic in tropical regions that populations are exposed to may have shaped the evolution of their

immune system to confer pro-inflammatory functions for protection. In doing so, this parasitic load may have driven selection for particular pro-inflammatory alleles/genotypes (135).

While the pro-inflammatory alleles/genotypes may be positively selected for in a tropical environment, they may have deleterious effects upon exposure to other or different environments. In more temperate conditions, for example, the profile of parasites and infectious diseases would differ and are less prevalent. Migration from a tropical to a more temperate environment, may result in the immune system being unnecessarily triggered to harmless environmental agents as a result of the pro-inflammatory alleles/genotypes. It has been shown that food allergy may be caused by increased inflammatory activation to environmental agents such as endotoxin (310). It is therefore plausible that genetic predisposition in those with tropical ancestry may have been masked by protective environmental factors. For instance, parasitic/helminthic infections have been shown to invoke large amounts of interleukin 10 (IL10), an anti-inflammatory cytokine, that dampen the immune responses to counterbalance the robust inflammatory immune system. Absence of such parasitic load would inhibit production of IL10, leaving a hyper-inflammatory immune response (311). Parasites and pathogens are also drivers of local adaptation, under natural selection. These pathogen-driven selection are found enriched in interleukin genes and genes involved in immune responses to parasitic worms (141, 190, 312). Additionally, *HLA* has also been implicated in such evolutionary pressures with an association between genetic diversity and pathogen richness at different loci of *HLA* (140). Together, these studies lend further support to the hypothesis that East Asians may be genetically predisposed to food allergy, combined with evidence showing East Asian children living in Melbourne at a higher risk of developing nut allergy than Australian children (49). In other words, the positively selected pro-inflammatory alleles/genotypes associated with helminths/parasites may be more common in those with long-term tropical ancestry (Asians) than those of temperate origin (Europeans/Caucasians).

Given the evidence of helminth driven selection for immune genotypes, we aimed to study the association between SNP at genes known to be associated with helminth-mediated immune responses, and food allergy in Asian and Caucasian populations in the HealthNuts cohort. Candidate gene approaches allow investigation of genetic variations

of multiple genes in a cost-effective manner. Although GWAS are more powerful in discovering novel susceptibility genes in a hypothesis-free approach, they often require a large sample size to be sufficiently powered to detect any associations (313). Given our specific hypothesis and a sample size too small for a well-powered GWAS, we decided on a candidate gene approach.

Therefore, the aim of this chapter is to develop a list of plausible candidate SNPs associated with helminth diversity, to genotype in samples obtained from the HealthNuts study. The results of the association testing between food allergy and these selected candidate SNPs will be presented in the next chapter (Chapter 7).

6.2 Research questions

1. Which helminth-related and/or allergy SNPs are suitable candidates for genotyping in the HealthNuts biological specimens, for future investigation of their association with food allergy?

6.3 Methods

Here, two main complementary strategies were used to curate a list of candidate SNPs. In brief, the first step involved a review of current literature on genes associated with food allergy and/or helminth diversity. In the next step, the set of candidate SNPs obtained from the review was narrowed down based on available genotyped data from our HealthNuts GWAS. Our GWAS was carried out on 796 participants but only a subset of the participants (n=221) were analysed in our published GWAS on peanut allergy. Further details on the data from HealthNuts GWAS used to curate the list of candidate SNPs are described below in Section 6.3.2. The condensed list of SNPs obtained from the second step was then used to design primers for genotyping.

6.3.1 Literature review

Two sets of literature reviews were carried out. The first was a systematic review on genetic association studies of food allergy which was presented previously in Chapter 5.

A second review was then carried out focusing on helminths-related SNPs with evidence of association with allergic diseases. In this review, a strategy similar to that followed for

the first systematic review on food allergy and its genetic associations, was applied to gather information more specifically on helminth-related polymorphisms. Relevant publications were sourced from the same databases (MEDLINE, Embase, PubMed) used in the systematic review. The search strategies used for the second literature review are shown in Tables 6.1 - 6.3. Polymorphisms were selected from relevant papers if they were associated with i) helminth diversity or ii) helminths and any allergy.

Table 6.1 Search strategy for Medline (Ovid)

No.	Search Query
1.	(hypersensitivity or allerg* or sensitisation).tw,kf,hw.
2.	Hypersensitivity/ge [Genetics]
3.	exp Genome-Wide Association Study/
4.	(gene*1 or genetic* or mutation* or polymorphism* or SNP* or allel* or genome or genomewide or genome-wide or GWAS).tw,kf,hw.
5.	exp Genes/
6.	exp Genetic Predisposition to Disease/ or exp Polymorphism, Single Nucleotide/
7.	Helminthiasis/im or Helminths/im or Necator americanus/im or Ascaris lumbricoides/im or Trichuris/im or Schistosomiasis/im or Enterobius/im or Malaria/im or Parasite/im or Parasitic Diseases/im or Cestoda/im
8.	Helminthiasis/cl or Helminths/cl or Necator americanus/cl or Ascaris lumbricoides/cl or Trichuris/cl or Schistosomiasis/cl or Enterobius/cl or Malaria/cl or Parasite/cl or Parasitic Diseases/cl or Cestoda/cl
9.	(tapeworm* or flatworm* or helminth* or trichuris or Schistosomiasis or malaria or parasit*).tw,kf,hw.
10.	Necator americanus.tw,kf,hw.
11.	Ascaris lumbricoides.tw,kf,hw.
12.	enterobius vermicularis.tw,kf,hw.
13.	(1 or 2) and (3 or 4 or 5 or 6) and (7 or 8 or 9 or 10 or 11 or 12)
14.	Limit 13 to (english language and humans and yr="2000 -Current")

Table 6.2 Search strategy for Embase (Ovid)

No.	Search Query
1.	exp allergy/
2.	(hypersensitivity or allerg* or sensitisation).tw,kw,hw.
3.	(gene*1 or genetic* or mutation* or polymorphism* or SNP* or allel* or genome or genomewide or genome-wide or GWAS).tw,kw,hw.
4.	exp gene/
5.	exp genome-wide association study/
6.	exp genetic predisposition/
7.	exp single nucleotide polymorphism/
8.	exp Helminthiasis/ or exp Helminths/ or exp Necator americanus/ or exp Ascaris lumbricoides/ or exp Trichuris/ or exp Schistosomiasis/ or exp Enterobius/ or exp Malaria/ or exp Parasite/ or exp Parasitic Diseases/ or exp Cestoda/
9.	(tapeworm* or flatworm* or helminth* or trichuris or Schistosomiasis or malaria or parasit*).tw,kw,hw.
10.	Necator americanus.tw,kw,hw.
11.	Ascaris lumbricoides.tw,kw,hw.
12.	enterobius vermicularis.tw,kw,hw.
13.	(1 or 2) and (3 or 4 or 5 or 6 or 7) and (8 or 9 or 10 or 11 or 12)
14.	limit 13 to (human and english language and yr="2000 -Current")

Table 6.3 PubMed search strategy

(tapeworm OR tapeworms OR flatworm OR flatworms OR helminths OR helminth OR helminthiasis OR trichuris OR schistosomiasis OR malaria OR parasite OR parasites OR parasitology OR “Necator americanus” OR “Ascaris lumbricoides” OR “enterobius vermicularis”) AND (allergy OR allergies OR hypersensitivities OR hypersensitivity OR sensitised OR sensitisation) AND (gene OR genes OR genetic* OR mutation* OR polymorphism* OR SNP OR SNPs OR allel* OR genome OR genomewide OR genome-wide OR “genome wide” OR GWAS) AND (NOTNLM OR publisher[sb] OR inprocess[sb] OR pubmednotmedline[sb] OR indatareview[sb] OR pubstatusaheadofprint)

Two separate sets of literature reviews had to be carried out because an initial search on genetic association studies of food allergy that have specifically investigated helminth-related polymorphisms did not garner enough papers.

6.3.2 Existing data from HealthNuts GWAS

The next in selecting candidate genes involved the use of existing data obtained from a GWAS carried out on samples obtained from the HealthNuts study (120). Samples were only available on a subgroup of HealthNuts participants who consented to the collection of biological specimens during assessments (refer to Chapter 2 Section 2.2). As such, only 764 samples were genotyped. Genotyping was carried out by Australian Genome Research Facility on Illumina HumanOmni 2.5-8 SNP array. Only unrelated individuals with genotyping call rate > 95% were retained for downstream analysis. The GWAS generated genotype data for 389 427 directly genotyped variants across the genome. These data were then used to impute unmeasured genetic variants resulting in a total of 3 814 967 SNPs post quality control. Quality control measures involved excluding SNPs that had MAF <1%, genotyping call rate <95% or Hardy Weinberg Equilibrium (HWE) test p-value < 10^{-6}, and/or had significant MAF differences when compared against the 1000 Genomes Project European samples ($p<0.001$).

Upon quality control, 496 individuals had high quality genotypes and the remaining 268 were excluded from further analyses. Of those 496, the published GWAS only carried out analyses on a subset of challenge-proven peanut allergics comprising of 221 individuals. These individuals were predominantly Europeans of which there were 73 cases of food allergy and 148 controls. In the published GWAS, cases were made up of 12-month old infants with a SPT wheal size of ≥ 2 mm above the negative control and/or peanut specific IgE > 0.35 kU/L on the day of oral food challenge and a positive reaction to an oral peanut challenge evident by clinical reactivity. The non-atopic control group comprised of 12-month old infants with a negative SPT to a panel of common food allergens (egg white, peanut, sesame, shrimp or cow's milk, cashew, almond, hazelnut, soya and wheat) and safely tolerated peanut during oral peanut challenge.

Since the GWAS in 2015, the HealthNuts study has followed up the same participants at ages 4 and 6 years (refer to Chapter 2 Section 2.1.2), therefore, we now have additional

biological samples collected from participants who did not consent to collection of biological samples in their previous visits, prior to the GWAS completion. This newly collected samples (n=206) alongside 268 samples which were included in the GWAS but did not pass quality control, were used for direct genotyping of the curated list of candidate SNPs.

To this end, from the list of SNPs obtained from literature review, only SNPs that were genotyped or imputed in the GWAS were selected as candidate SNPs for downstream targeted genotyping in the newly collected biological samples. Candidate SNPs were restricted to those captured in the GWAS to build on the existing data and improve sample size by directly genotyping a subset of the GWAS SNPs in newly collected biological samples. In particular, the GWAS did not include any analyses on participants with Asian background which can be further utilised and is the focus of the analyses included in this thesis. A downside to such an approach, however, is the possibility of missing key SNPs that may not have been captured in the GWAS. Further details on the samples that were genotyped, genotyping processes and analyses carried out in these samples can be found in the next chapter, Chapter 7.

6.4 Results and Conclusion

On top of the first literature review carried out (see Chapter 5), a second literature review carried out on association with helminths diversity, collated 81 potentially relevant articles and had their full text reviewed. Of those, 15 articles were deemed relevant and information on investigated SNPs were noted from each of the 15 studies. Collectively, from the two literature reviews carried out, a total of 112 SNPs annotated to 48 genes were selected. From the 112 SNPs, only SNPs that had been genotyped or imputed in the GWAS were chosen. This was a basis of the selection criteria as the aim was to improve the sample size and build on existing data available in the HealthNuts study. Consequently, 46 SNPs corresponding to 28 genes remained - 18 SNPs were directly genotyped by SNP array in the GWAS while the remaining 28 SNPs were imputed from the array data. A summary of the strategies used and the number of SNPs selected at each step is provided in Figure 6.1.

The 46 SNPs selected for genotyping were from the following genes *ADAM17, CHIA, IL10RA, PTGER2, TLR4, DPP10, CHL1, TYRP1, ADRB2, CD200R1L, CTLA4, EDAR, GATA3, HLA, IFNG, IKZF2, IL4, IL4R, IL6, IRS2, KCNS3, LIG4, NPSR1, PHF11, SLC39A8, SOCS5, STAT5B, TGFb1* (Table 6.4). The majority of these genes have been implicated in helminth diversity and are involved in immunity and regulation of inflammation. Interestingly, one of the genes, *EDAR* is known to be associated with hair thickness and has been shown to be positively selected for in the Asian population (314, 315).

These 46 SNPs were uploaded to an Agena Bioscience Assay Design Suite software (https://agenacx.com/) to design primers for genotyping. Consequently, only 37 SNPs were successfully incorporated into the design. Details on the assay design for the 37 SNPs included for genotyping is provided in the next chapter, Chapter 7.

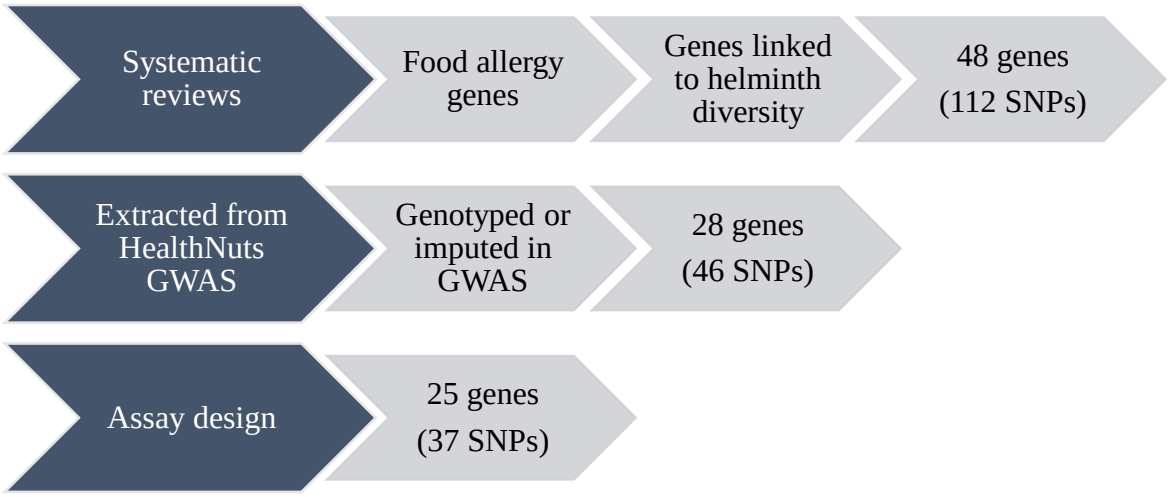

Figure 6.1 Pipeline for selecting candidate genes and SNPs for genotyping. In summary, two literature reviews on the relevant topics were carried out giving a total of 112 SNPs. These SNPs were extracted from an existing GWAS in HealthNuts giving rise to 46 SNPs that were either imputed or genotyped directly on the SNP array in the GWAS. Of the 46 SNPs, 37 SNPs were successfully incorporated into the assay design for genotyping using HealthNuts samples.

Table 6.4 List of 46 SNPs input into assay design for genotyping

					1000G EAS		1000G EUR			
SNP	**CHR**	**Gene annotated to in literature**	**Reference allele**	**Alternate allele**	**MAF reference allele**	**MAF alternate allele**	**MAF reference allele**	**MAF alternate allele**	**GWAS[a]**	**Investigated outcome in literature**
rs10494133	1	*CHIA*	T	C	1	0	0.891	0.109	Imputed	IgE levels to both Ascaris and common allergens (316)
rs1056204	2	*ADAM17*	A	C	0.997	0.003	0.68	0.32	Genotyped	Helminth diversity (141)
rs10495562	2	*ADAM17*	T	C	0.979	0.021	0.489	0.511	Genotyped	Helminth diversity (141)
rs7579207	2	*DPP10*	A	G	0.023	0.977	0.306	0.694	Genotyped	Helminth diversity, allergy (141)
rs231735	2	*CTLA4*	G	T	0.267	0.733	0.516	0.484	Imputed	Helminth diversity, allergy (141)
rs11571291	2	*CTLA4*	T	C	0.732	0.268	0.555	0.445	Imputed	Helminth diversity, allergy (141)
rs231804	2	*CTLA4*	C	T	0.267	0.733	0.451	0.549	Imputed	Helminth diversity, allergy (141)
rs4353658	2	*DPP10*	G	A	0.854	0.146	0.55	0.45	Genotyped	Helminth diversity, allergy (141)
rs6749207	2	*EDAR*	T	C	0.192	0.808	0.855	0.145	Imputed	Helminth diversity, hair thickness (141)
rs12619285	2	*IKZF2*	A	G	0.367	0.633	0.746	0.254	Imputed	Helminth diversity, allergy (141)

					1000G EAS		1000G EUR			
SNP	**CHR**	**Gene annotated to in literature**	**Reference allele**	**Alternate allele**	**MAF reference allele**	**MAF alternate allele**	**MAF reference allele**	**MAF alternate allele**	**GWAS[a]**	**Investigated outcome in literature**
rs4368333	2	*KCNS3*	C	A	0.945	0.055	0.432	0.568	Imputed	Helminth diversity, allergy (141)
rs6737848	2	*SOCS5*	C	G	0.594	0.406	0.926	0.074	Imputed	Atopic bronchial asthma (317)
rs4684083	3	*CHL1*	T	C	0.951	0.049	0.745	0.255	Genotyped	Helminth diversity (141)
rs4682429	3	*CD200R1L*	G	A	0.901	0.099	0.558	0.442	Genotyped	Helminth diversity (141)
rs11096956	4	*IL4R*	C	A	0.567	0.433	0.75	0.25	Imputed	Self-reported allergy and Helicobacter pylori serologic status (318)
rs10004195	4	*IL4R*	T	A	0.519	0.481	0.721	0.279	Imputed	Self reported allergy and Helicobacter pylori serologic status (318)
rs10024216	4	*IL4R*	G	A	0.433	0.567	0.608	0.392	Imputed	Self reported allergy and Helicobacter pylori serologic status (318)
rs10014145	4	*SLC39A8*	A	G	0.876	0.124	0.675	0.325	Genotyped	Helminth diversity (141)
rs877741	5	*ADRB2*	T	C	0.215	0.785	0.812	0.188	Genotyped	Helminth diversity, allergy (141)

					1000G EAS		1000G EUR			
SNP	**CHR**	**Gene annotated to in literature**	**Reference allele**	**Alternate allele**	**MAF reference allele**	**MAF alternate allele**	**MAF reference allele**	**MAF alternate allele**	**GWAS[a]**	**Investigated outcome in literature**
rs2243290	5	*IL4*	C	A	0.217	0.783	0.83	0.17	Genotyped	Helminth diversity (141)
rs2243268	5	*IL4*	A	C	0.224	0.776	0.833	0.167	Genotyped	Helminth diversity, allergy (141)
rs12186803	5	*KIF3A/IL4*	G	A	0.247	0.753	0.831	0.169	Imputed	Helminth diversity (141)
rs2243250	5	*IL4*	C	T	0.221	0.779	0.832	0.168	Imputed	Th2-predominant immune response (319)
rs2070874	5	*IL4*	C	T	0.221	0.779	0.832	0.168	Genotyped	Helminth diversity (141)
rs7192	6	*HLA*	T	G	0.313	0.687	0.367	0.633	Genotyped	Peanut allergy (118)
rs2066992	7	*IL6*	G	T	0.212	0.788	0.952	0.048	Genotyped	Micro- and macro-pathogen richness (312)
rs10237930	7	*NPSR1*	T	C	0.731	0.269	0.504	0.496	Imputed	Helminth diversity, allergy (141)
rs1927936	9	*TLR4*	C	A	0.993	0.007	0.717	0.283	Imputed	Helminth diversity (141)
rs7849955	9	*TLR4*	G	A	0.998	0.002	0.854	0.146	Imputed	Helminth diversity, allergy (141)
rs1927937	9	*TLR4*	A	G	0.991	0.009	0.717	0.283	Imputed	Helminth diversity (141)

					1000G EAS		1000G EUR			
SNP	**CHR**	**Gene annotated to in literature**	**Reference allele**	**Alternate allele**	**MAF reference allele**	**MAF alternate allele**	**MAF reference allele**	**MAF alternate allele**	**GWAS[a]**	**Investigated outcome in literature**
rs1927934	9	*TLR4*	T	C	0.993	0.007	0.717	0.283	Imputed	Helminth diversity (141)
rs1930713	9	*TLR4*	G	A	0.957	0.043	0.736	0.264	Genotyped	Helminth diversity, allergy (141)
rs2245960	9	*TLR4*	C	T	0.956	0.044	0.768	0.232	Imputed	Helminth diversity, allergy (141)
rs1952692	9	*TYRP1*	A	C	0.954	0.046	0.668	0.332	Imputed	Helminth diversity (141)
rs10905349	10	*GATA3*	A	G	0.525	0.475	0.165	0.835	Genotyped	Helminth diversity, allergy (141)
rs3020913	11	*IL10RA*	A	G	0.98	0.02	0.687	0.313	Genotyped	Helminth diversity (141)
rs2512144	11	*IL10RA*	A	G	0.007	0.993	0.329	0.671	Imputed	Micro- and macropathogen richness (312)
rs2069705	12	*IFNG*	G	A	0.757	0.243	0.327	0.673	Imputed	Atopic bronchial asthma (317)
rs2289046	13	*IRS2*	T	C	0.535	0.465	0.69	0.31	Imputed	Total IgE levels in asthmatics (316)
rs1805388	13	*LIG4*	G	A	0.79	0.21	0.837	0.163	Imputed	IgE levels to *Ascaris* (316)
rs7329078	13	*PHF11*	T	C	0.192	0.808	0.403	0.597	Imputed	Helminth diversity, allergy (141)
rs708491	14	*PTGER2*	A	G	0.993	0.007	0.72	0.28	Genotyped	Helminth diversity, allergy (141)

					1000G EAS		1000G EUR			
SNP	**CHR**	**Gene annotated to in literature**	**Reference allele**	**Alternate allele**	**MAF reference allele**	**MAF alternate allele**	**MAF reference allele**	**MAF alternate allele**	**GWAS[a]**	**Investigated outcome in literature**
rs16967593	17	*STAT5B*	T	A	0.636	0.364	0.719	0.281	Imputed	Atopic bronchial asthma (317)
rs1800469	19	*TGFb1*	A	G	0.547	0.453	0.312	0.688	Genotyped	Helminths infections, allergy (320)
rs1800470	19	*TGFb1*	G	A	0.555	0.445	0.382	0.618	Imputed	Helminths infections, allergy (320)
rs2241712	19	*TGFb1*	C	T	0.554	0.446	0.325	0.675	Imputed	Helminths infections, allergy (320)

Single nucleotide polymorphism (SNP); Chromosome (CHR); Minor allele frequency (MAF); East Asian population (EAS), European population (EUR), 1000 genomes project (1000G); Genome wide association studies (GWAS)

[a] Column refers to whether genotype data for SNP extracted from HealthNuts GWAS was obtained via SNP array imputation or direct genotyping of samples

Chapter 7 Genetic risk factors for food allergy

7.1 Introduction

This chapter builds on the argument for the role of helminth-driven selection in immune genotypes as presented in Chapter 6. Tropical populations exposed to an environment with high parasitic load may have developed a pro-inflammatory immune system. While conferring protection against infections, the pro-inflammatory immune system conversely constitutes a risk factor for allergic diseases. We hypothesised that genetic variants with pro-inflammatory functions may be more frequent in those with long-term tropical ancestry (Asians) than those of temperate origin (Caucasians).

Using the list of candidate genes outlined in the previous chapter, this chapter aims to examine the association between those SNPs and food allergy in Asian and Caucasian populations in the HealthNuts cohort.

7.2 Research questions

This chapter will answer the following research questions:

1. Are helminth-related SNPs associated with food allergy in the East Asian population living in Australia?
2. Are these SNPs also associated with food allergy in the Caucasian population living in Australia?

7.3 Methodology

To address the research question, data obtained from the HealthNuts study was used. The HealthNuts study methods have been described in detail in Chapter 2, Section 2.2.

7.3.1 Study cohort

Briefly, baseline data were collected at 12 months where infants were recruited at community immunisation centres (N=5,276). Infants underwent skin prick test to four main foods (egg, peanut, sesame, shrimp/cow's milk) at the immunisation centres. Infants with

a detectable wheal size ≥ 1 mm to any of the foods were invited to the HealthNuts clinic at the Royal Children's Hospital, Melbourne. During the clinic visit, infants underwent a repeated skin prick test and blood test to determine their food specific IgE level. Infants found to be sensitised were then offered an OFC to ascertain their food allergy status. OFCs were carried out using a pre-determined protocol described earlier in Chapter 2. The following objective criteria were used to define a positive OFC: i) three or more concurrent non-contact urticaria persisting for at least five minutes; ii) perioral or periorbital angioedema; iii) vomiting and/or iv) evidence of circulatory or respiratory compromise, occurring within two hours of ingestion of a dose during OFC. The OFC was deemed negative if the infant was able to complete the challenge with no reaction (157).

7.3.1.1 Collection of biological samples

We collected venepuncture blood during the participants' visit into the clinic and had access to newborn screening cards for those who consented to it. Details on processing of biological specimens are presented in Chapter 2, Section 2.3. DNA was extracted from these samples (regardless of the age at which the samples were obtained) for downstream SNP genotyping. Details of DNA extractions for each type of sample are provided in Chapter 2, Section 2.4.

7.3.2 SNP selection

A multi-step approach was undertaken to curate a list of candidate SNPs to genotype. The process of candidate SNPs selection has been detailed in Chapter 6. In total, 37 SNPs were selected for assay design.

7.3.2.1 Assay design and primer sequences

Primer sequences for genotyping were designed using the Agena Bioscience Assay Design Suite software package https://agenacx.com/ (321). This assay design platform ensures the highest possible multiplex level (up to 40 SNPs per well) is achieved without compromising sensitivity and consistency of results. The assay design identifies PCR amplification primers that will result in only a unique amplification product and minimises any possible primer dimer formation and interactions that could lead to false positive signals.

Of the 46 SNPs extracted from the HealthNuts GWAS (see Chapter 6), nine SNPs were eventually excluded from inclusion in assay design. Six of those excluded had a MAF of less than 0.05 in the 1000 Genome East Asian population, indicating that they have low heterozygosity and will therefore be infrequently detected in a small sample size. Three other SNPs were removed as data on the Single Nucleotide Polymorphism Database (dbSNP) showed that the three SNPs were not biallelic. Based on past experience of genotyping non-biallelic SNPs, such SNPs proved to be difficult to cluster using the allele calling method of the system software (MassARRAY®) used in the targeted genotyping approach. Therefore, these three non-biallelic SNPs were excluded from the assay design. Details including MAF from 1000 Genomes and alleles of each of the remaining 37 SNPs included in the assay design shown in Appendix 7.

All primers were obtained from Integrated DNA Technologies (Coralville, Iowa, USA), a commercial manufacturer of oligonucleotides. Lyophilized primers were reconstituted with MilliQ water to their respective stock concentrations and stored frozen. The forward and reverse primers were ordered at 25 nM and reconstituted to 100 μM while the extension primers were ordered at 100 nM and reconstituted to 500 μM. The PCR primers were pooled into a stock primer mix at a concentration of 0.5 μM for each primer. The concentrations of extension primers were adjusted to equilibrate the signal-to-noise ratios and produce approximately equal peak intensities across all extension primers. Primer adjustment were determined through a gradient algorithm (available from Agena Bioscience).

The final assay design resulted in two separate pools of primers – Assay 1 with 27 SNPs and Assay 2 with 10 SNPs. Primers for these two assays are detailed in Appendix 8. Given the sheer number of genes mentioned in this chapter, the abbreviated gene names will be used instead of the full description. Details on full description of gene names used in this chapter and thesis can be found in Appendix 9.

7.3.3 Genotyping and quality control

7.3.3.1 Genotyping

Genomic DNA from venepuncture blood samples and newborn screening cards (n=485) were genotyped using the iPLEX™ Gold chemistry on the Sequenom (now Agena Bioscience) MassARRAY® MALDI-TOF mass spectrometer as described in Chapter 2, Section 2.5. In short, DNA amplification was first carried out through PCR to amplify the target regions. This was followed by a SAP purification step, which neutralises any unincorporated nucleotides. In the final step, iPLEX Gold chemistry was used. iPLEX Gold reaction was made up of a mix of oligonucleotide extension primers, extension enzyme and mass-modified dideoxynucleotide terminators. The extension primers annealed directly adjacent to each SNP site to be assayed, and were extended and terminated by a single complementary base into the genotyping target site. The extension products were then desalted using clean resin before being transferred onto a chip array by the MassARRAY® Nanodispenser (described in detail in Chapter 2, Section 2.5.4).

7.3.3.2 Firing and calling

Once the samples have been transferred onto the chip, the chip was placed into a mass spectrometer to determine the mass of the DNA fragments. Under vacuum conditions, each spot on the chip was fired at with UV laser light and the time taken for each sample analyte to travel from the bottom of the mass spectrometer to the top corresponds to the mass of the extended primer (163). The MALDI-TOF mass spectrometry system then correlates the mass of the extended base to the allele present at the polymorphic site. Data obtained was reflected onto the software SpectroTYPER V4.0. An example of spectrum obtained from samples reflecting the alleles and intensity peaks in SpectroTYPER is shown in Figure 7.1.

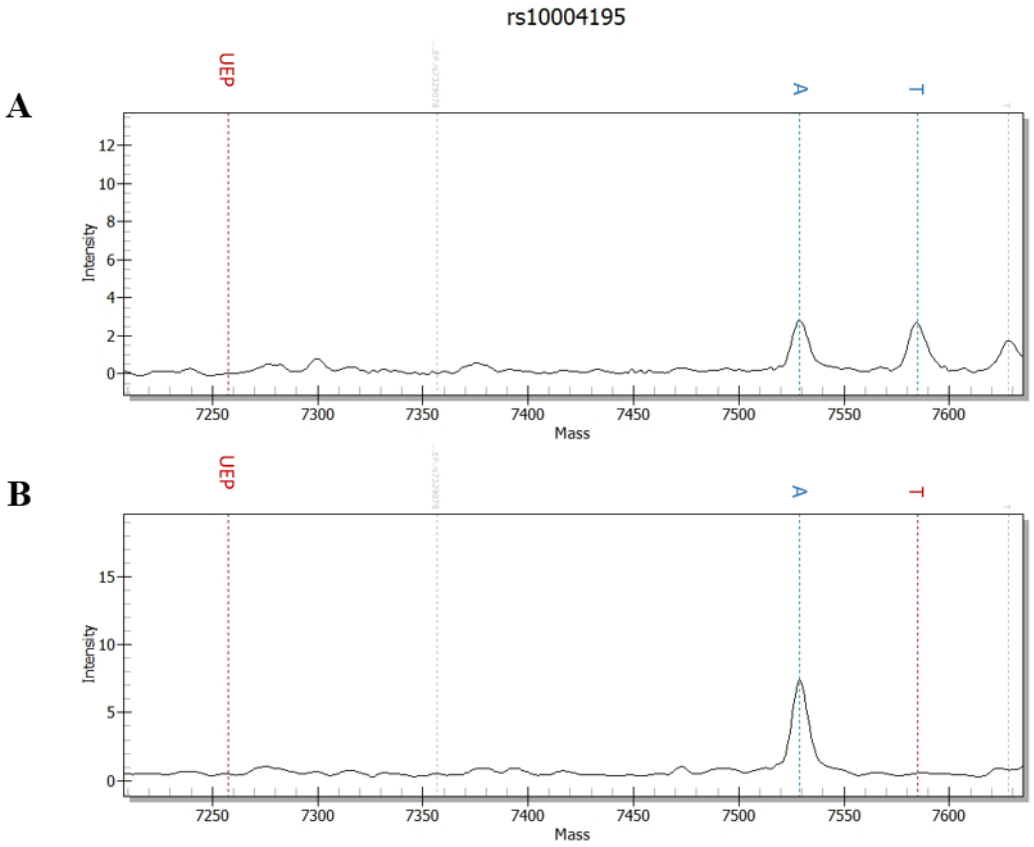

Figure 7.1 Spectrum of heterozygote and homozygote sample of rs10004195. A) For heterozygotes, the peaks of two alleles, A and T, are clearly marked on the spectrum. The position of the peak for unincorporated extension primer (UEP) is reflected on the spectrum as well. B) For homozygotes, only one peak, representing the A allele, is reflected on the spectrum. In both A) and B), the low peak heights of the UEP indicate that little UEP is left and the UEP was successfully used in the iPLEX extension reaction.

7.3.3.3 Quality control

Quality control checks of MassARRAY® genotyped data in SpectroTYPER software V4.0 was done through visual inspection of the mass spectra cluster plots. This assists in the identification of errors in automated calling and samples that have failed the assay or generated poor quality data. Poorly performing SNPs with potential errors or those that were not clustering well were discarded first. Thereafter, poorly performing samples with less than 90% calling rate were excluded from analyses.

To detect potential genotyping error, Hardy Weinberg Equilibrium (HWE) testing was also carried out for each SNP. SNPs with a significant deviation from the expected equilibrium, indicated by $P \leq 0.01$ were also removed from analyses.

7.3.4 Definitions

Ancestry background: Ancestry background was defined based on parent's country of birth which has been shown to be a good proxy marker as detailed in Chapter 2, Section 2.1.4. ***East Asians*** consisted of children with both parents born in North East Asia region such as China, Hong Kong, Japan, Taiwan, Macau, North Korea, South Korea and South East Asia region of Vietnam, Philippines, Singapore, Thailand, Indonesia, Malaysia, Cambodia, Laos. ***Admixed*** population consisted of infants with one parent born in East Asia. ***Caucasians*** are children with parents born in Australia, New Zealand, UK, Europe or North America. These countries were grouped based on the Standard Australian Classification of Countries (SACC) which were developed to be relevant to Australia's multicultural society for use in analysing Australian-based country of origin data (161). Groups in the SACC comprise geographically proximate countries which have broadly similar social, cultural, economic and political characteristics.

Non-sensitised, non-allergic controls: Individuals were classified as controls if they are negative to SPT to egg, peanut or sesame.

Food sensitised tolerant: Food sensitisation was defined as the presence of specific IgE to a food allergen. Sensitised individuals could be either allergic (experience symptoms on consumption of the food in question) or tolerant (able to consume the food without reaction). Infants were classified as food sensitised tolerant if their SPT was greater or equal to 2 mm to at least one of the following food (egg, peanut or sesame) on the day of OFC in the context of a negative saline control and positive histamine control or positive CAP-FEIA (0.35 kU/L) to the food in question measured during the OFC clinic.

Food sensitised allergics: Infants were classified as having food allergy if they had a positive SPT and positive OFC to egg, peanut or sesame.

7.3.5 Statistical analysis

7.3.5.1 Combining MassARRAY® genotyped data with existing HealthNuts GWAS data

As described in Chapter 6, candidate SNPs investigated in this chapter were a subset of those measured in a GWAS previously carried out for peanut allergy in a Caucasian population of the HealthNuts study. Genotyped data for the candidate SNPs were extracted from the GWAS Illumina HumanOmni 2.5-8 SNP array for samples where existing GWAS data were available. Extracted SNPs were not limited to those directly genotyped on the SNP array but also included those inferred by imputation. Thereafter, combined analyses were carried out on the extracted GWAS data (n=495) and MassARRAY® genotyped data (n=485). A breakdown of the source of biological sample for DNA extractions and the corresponding number of samples in each ancestry group is shown in Figure 7.2.

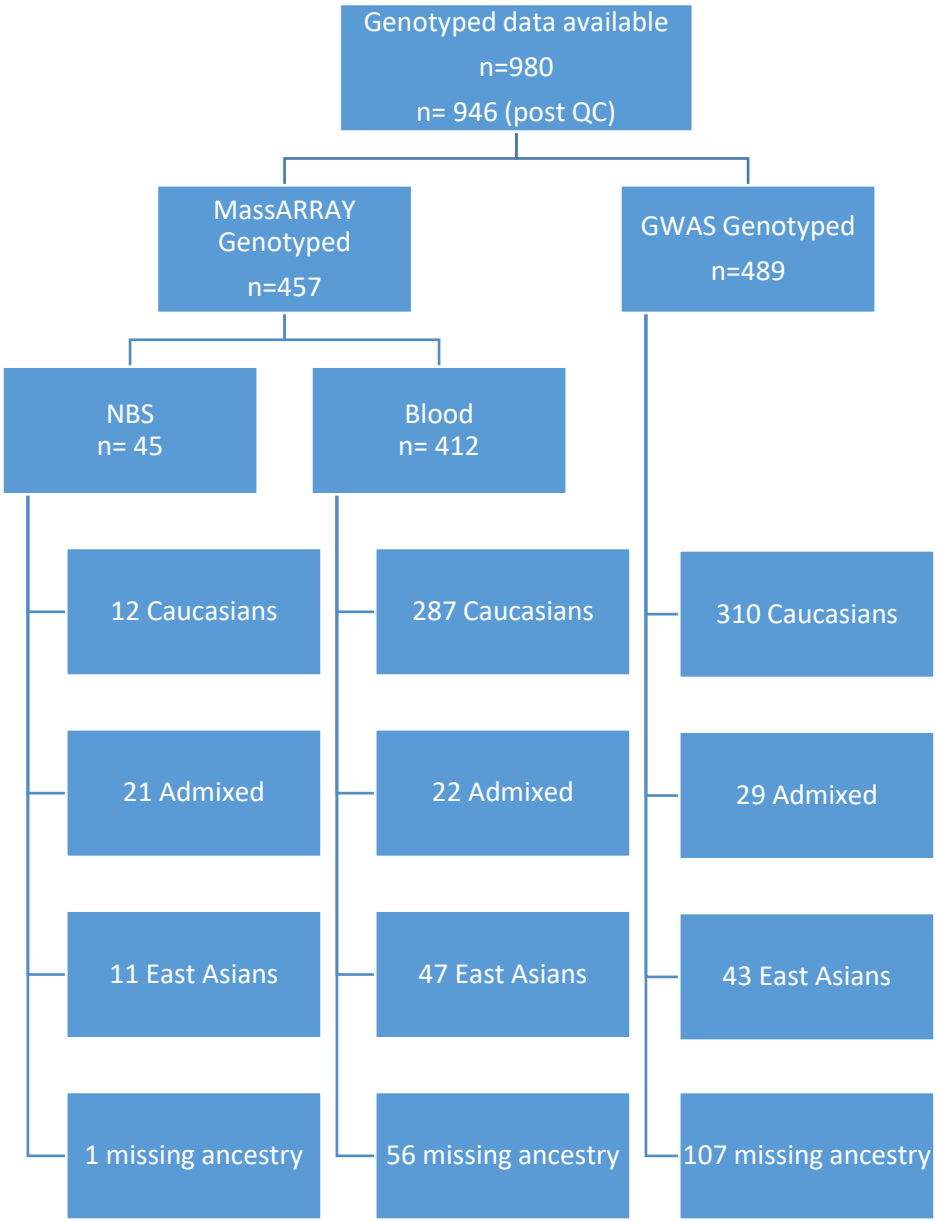

Figure 7.2 Flowchart on the source of data and samples used in analyses. Genotyped data were available on 946 samples post-quality control. Of the 457 DNA samples genotyped using MassARRAY®, 45 were extracted from newborn screening cards (NBS) and the remaining 412 were extracted from venepuncture blood samples. The remaining 489 genotyped data post-quality control were extracted from the HealthNuts genome wide association study (GWAS).

7.3.5.2 Genetic association analysis

In genetic association analyses for a case-control study, an association exists between genetic variant and phenotype if the frequencies of alleles or genotypes of investigated SNPs differ between the cases and controls. Statistical association tests based on different allelic or genotypic models can be carried out to assess evidence for association.

In this chapter, the main analysis compared allele frequencies between cases and controls using the basic allelic test in PLINK (V1.90), a whole genome association analysis tool. As an illustration, given a minor allele A and major allele T for a particular SNP, the basic allelic test carries out a frequency analysis for association with food allergy - frequency of A allele in cases vs frequency of A allele in controls.

For SNPs that showed some evidence of association (nominal $P < 0.1$), several other models were also carried out to investigate whether the risk of food allergy conferred by the particular SNP might act via a different trait model. The genetic model of action underlying the association between genotype and food allergy is still unclear. Therefore, analyses of these alternative models will help increase the power of detecting an association using the model that best fits the potential mechanism of action. These alternative trait models include the additive (Cochrane-Armitage test for trend), dominant, recessive and genotypic trait models.

The additive model assumes that carriers of two risk alleles have an increased risk compared to those having a single copy who in turn have an increased risk compared to those with no risk allele. Using the same example above, given that minor allele A is also the risk allele, the additive model would assume that carriers of AA have around twice the risk of expressing the phenotype than carriers of AT.

The dominant model assumes that an effect on phenotype is seen when there is at least one copy of the risk allele (i.e AA and AT vs TT). Conversely, the recessive model assumes that an effect on phenotype is only seen in the presence of two copies of the minor allele (i.e. AA vs AT or TT). On the other hand, genotypic model assumes different disease risks based on different genotypes AA vs AT vs TT.

These analyses were repeated for each of the three population groups (Caucasian infants, Admixed infants, East Asian infants). An additional sub-analysis was carried out in a combined group of East Asian comprising of infants with **one or both** parents born in East Asia.

The primary outcome of this chapter is 'any food allergy' where the non-atopic controls were compared to the food allergic infants. Analyses were also carried out for several secondary outcomes listed below:

- Peanut allergy (non-atopic controls vs peanut allergics)
- Egg allergy (non-atopic controls vs egg allergics)
- Sensitisation to any food (non-atopic controls vs food sensitised tolerant)
- Peanut sensitisation (non-atopic controls vs peanut sensitised tolerant)
- Egg sensitisation (non-atopic controls vs egg sensitised tolerant)
- Egg sensitised tolerant vs Egg allergics
- Peanut sensitised tolerant vs Peanut allergics
- Food sensitised tolerant vs Food allergics

Stata/IC 15 software was used for all other statistical and demographic analyses.

Significance was defined at $p < 0.05$ (after Bonferroni correction) and obtained from PLINK. Given the number of tests (n=35 SNPs) carried out in this chapter, correction for multiple testing using tests such as Bonferroni correction was deemed essential to minimise false-positive associations arising due to chance.

7.3.5.3 Power calculation

Power calculations were computed using QUANTO (available from http://biostats.usc.edu/Quanto.html), a software for determining statistical power and sample sizes in genetic and gene-environment interaction studies. The study was predicted to have sufficient power to detect effect sizes over 1.5 at an alpha level of 0.8 for SNPs with MAF greater than 0.05 in the Caucasian population. In the Asian population, sufficient power at an alpha level of 0.80 were predicted at effect sizes over 1.8.

7.4 Results

Of the 37 SNPs genotyped by MassARRAY®, two SNPs, rs1800469 and rs10237930 had poor separation of heterozygotes from the minor/major allele homozygotes cluster and were excluded from further analyses (Figure 7.3). The remaining 35 SNPs genotyped did not deviate from HWE and were included in analyses. The MAFs and HWE values for each of the 35 SNPs are shown in Table 7.1.

Of the 980 genotyped individuals available, 946 were retained for analyses after removing 34 samples which had less than 90% calling rate. Data points included in analyses post-quality control consisted of 346 non-sensitised non-allergic infants, 75 food sensitised tolerant infants and 382 food sensitised allergic infants. The remaining 143 samples either had missing food allergy status or ancestry information. As a result, only 748 samples had both food allergy status **and** ancestry information.

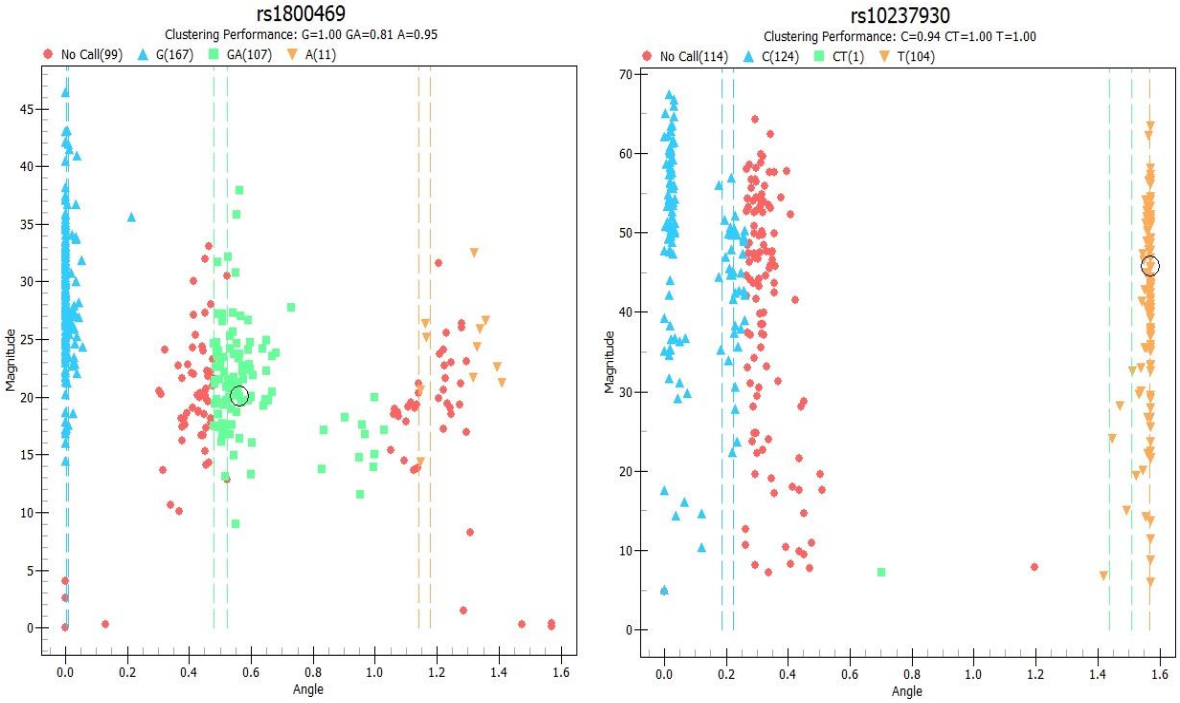

Figure 7.3 Screenshot of calls from SpectroTYPER software for rs1800469 and rs10237930. Each blue triangle denotes an individual with a homozygous minor/major allele for that particular single nucleotide polymorphism (SNP). Each green circle represents a heterozygous individual while each orange triangle represent an individual with a homozygous major/minor allele. Samples with no call data are marked red. For both

SNPs, rs1800469 and rs10237930, there were no clear separation between the minor/major homozygotes and heterozygotes. There was an overlap of calls that were marked as either heterozygotes or homozygotes despite similar peak heights. These suggest that it cannot be confirmed with certainty that these calls were accurate and therefore were removed from analyses.

Table 7.1 Minor allele frequency (MAF) and Hardy-Weinberg equilibrium (HWE) values of 35 SNPs investigated for an association with food allergy.

	Caucasians (N=624)			Admixed (N=77)			East Asians (N=109)		
SNP	**MAF**	**HWE whole sample**	**HWE Con-trols**	**MAF**	**HWE whole sample**	**HWE Con-trols**	**MAF**	**HWE whole sample**	**HWE Con-trols**
rs10495562	0.527	0.684	0.231	0.181	0.106	0.575	0.050	1.000	1.000
rs1056204	0.307	0.390	1.000	0.090	1.000	1.000	0.010	1.000	1.000
rs4368333	0.559	0.564	1.000	0.261	0.125	0.066	0.059	0.293	1.000
rs6737848	0.092	0.628	0.740	0.293	0.773	0.684	0.439	0.423	0.345
rs6749207	0.155	0.212	0.052	0.565	0.461	0.204	0.740	0.602	0.032
rs4353658	0.395	0.672	0.360	0.354	0.797	0.732	0.145	1.000	0.463
rs7579207	0.321	0.781	0.455	0.153	0.199	0.045	0.030	1.000	1.000
rs231735	0.459	0.041	0.104	0.368	0.803	0.316	0.237	0.091	1.000
rs231804	0.414	0.034	0.215	0.326	0.591	1.000	0.230	0.148	1.000
rs11571291	0.415	0.111	0.144	0.310	1.000	1.000	0.235	0.055	1.000
rs12619285	0.259	0.396	0.095	0.457	0.629	0.748	0.580	0.546	0.527
rs4684083	0.306	0.104	0.174	0.132	1.000	1.000	0.060	1.000	1.000
rs4682429	0.432	0.620	0.582	0.257	0.766	0.652	0.089	0.564	0.307
rs10024216	0.353	0.531	0.906	0.471	0.633	0.348	0.479	0.839	0.760
rs10004195	0.248	0.582	0.392	0.418	1.000	1.000	0.429	0.398	0.323
rs10014145	0.316	0.570	0.900	0.204	0.465	0.564	0.139	1.000	0.380
rs2243250	0.184	0.021	0.455	0.549	1.000	1.000	0.772	0.392	0.696
rs2070874	0.183	0.029	0.569	0.549	0.816	1.000	0.772	0.392	0.696
rs2243268	0.182	0.014	0.341	0.549	1.000	1.000	0.772	0.392	0.696
rs2243290	0.185	0.021	0.454	0.569	0.633	1.000	0.767	0.405	0.697

	Caucasians (N=624)			Admixed (N=77)			East Asians (N=109)		
rs12186803	0.174	0.023	0.436	0.549	0.816	0.751	0.745	0.433	0.721
rs877741	0.209	1.000	0.867	0.478	0.628	0.738	0.755	0.012	0.173
rs7192	0.374	0.863	0.818	0.401	0.807	1.000	0.282	0.460	0.726
rs1952692	0.347	0.788	1.000	0.183	0.439	1.000	0.056	1.000	1.000
rs7849955	0.141	0.615	1.000	0.069	1.000	1.000	0.020	1.000	1.000
rs1930713	0.233	0.140	1.000	0.139	0.339	0.564	0.045	1.000	1.000
rs2245960	0.181	0.495	0.108	0.118	0.583	1.000	0.040	1.000	1.000
rs10905349	0.204	0.619	0.389	0.347	1.000	0.717	0.525	0.843	0.532
rs2069705	0.323	0.189	0.262	0.586	0.627	0.748	0.697	0.160	0.694
rs7329078	0.397	0.733	0.135	0.261	0.764	0.691	0.258	0.599	0.287
rs1805388	0.146	0.621	0.171	0.203	0.130	0.140	0.218	0.012	0.084
rs2289046	0.323	0.266	0.899	0.403	1.000	1.000	0.515	0.689	0.763
rs708491	0.267	0.006	0.086	0.072	1.000	1.000	0.020	1.000	1.000
rs16967593	0.298	0.286	0.624	0.285	0.149	1.000	0.400	0.532	0.340
rs2241712	0.348	0.232	1.000	0.416	0.630	0.513	0.542	0.681	0.753

SNP – single nucleotide polymorphism, MAF – minor allele frequency, HWE – Hardy Weinberg equilibrium

7.4.1 *STAT5B* variant is associated with a reduced risk of any food allergy

To assess whether any of the genetic variants tested are associated with any food allergy (egg, peanut or sesame), the non-sensitised, non-allergic control infants (negative controls) (total n=346) were compared to the food sensitised allergic infants (total n=382) in each of the population group.

There were no SNPs associated with any food allergy that were common among the three ancestry populations. Among the Caucasian infants, variant rs16967593 (*STAT5B*) was associated with reduced odds of any food allergy (P = 0.00133; OR 0.65 CI 0.49-0.84; P_{adjusted} =0.047). This variant was also associated with food allergy in all other trait models tested ($P_{unadjusted}$ <0.05) (Table 7.2).

In the admixed population group, three other variants - rs6749207 (*EDAR*), rs12186803 (*IL4*) and rs1805388 (*LIG4*) were found to have weak associations with any food allergy prior to multiple testing adjustment (Table 7.2). The rs6749207 ($P_{unadjusted}$=0.012) and rs12186803 ($P_{unadjusted}$ =0.027) were both associated with food allergy when modelled as an additive trait.

None of the investigated SNPs were associated with food allergy, either before or after multiple testing adjustment, in infants with two East Asian-born parents.

Table 7.2 Association of investigated SNPs with 'any food allergy' by parent's country of birth (non-atopic controls vs food allergic cases)

			Caucasians (n=519)						Admixed (n=64)						East Asian (n=90)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
ADAM17	2	rs10495562	T	0.49	0.637	1.06	0.83	1.35	C	0.20	0.900	1.06	0.44	2.54	C	0.04	0.555	0.65	0.16	2.70
ADAM17	2	rs1056204	C	0.31	0.613	1.07	0.82	1.40	C	0.12	0.595	1.37	0.43	4.31	C	0.01	0.772	0.66	0.04	10.78
KCNS3	2	rs4368333	C	0.43	0.361	0.89	0.70	1.14	A	0.20	0.132	0.54	0.24	1.21	A	0.06	0.703	0.79	0.23	2.69
SOCS5	2	rs6737848	G	0.09	0.584	1.13	0.73	1.73	G	0.31	0.398	1.41	0.64	3.10	G	0.44	0.602	1.18	0.64	2.16
EDAR	2	rs6749207	C	0.15	0.954	0.99	0.70	1.39	T	0.31	**0.018 A** 0.614 B **0.012 T**	0.41	0.19	0.86	T	0.27	0.726	1.13	0.57	2.24
DPP10	2	rs4353658	A	0.41	0.262	1.15	0.90	1.48	A	0.37	0.737	1.13	0.55	2.34	A	0.15	0.922	1.04	0.44	2.45
DPP10	2	rs7579207	A	0.31	0.961	1.01	0.77	1.31	A	0.15	0.855	0.91	0.35	2.39	A	0.05	0.235	3.45	0.39	30.13
CTLA4	2	rs231735	G	0.46	0.936	0.99	0.78	1.26	G	0.37	0.855	0.94	0.46	1.92	G	0.20	0.480	0.77	0.37	1.59
CTLA4	2	rs231804	C	0.42	0.751	1.04	0.81	1.34	C	0.33	0.906	1.05	0.50	2.19	C	0.18	0.337	0.70	0.33	1.46
CTLA4	2	rs11571291	C	0.44	0.394	1.11	0.87	1.43	C	0.29	0.713	0.87	0.41	1.85	C	0.20	0.544	0.80	0.39	1.65
IKZF2	2	rs12619285	G	0.23	0.291	0.86	0.64	1.14	G	0.45	0.811	0.92	0.45	1.86	A	0.39	0.559	0.83	0.45	1.53
CHL1	3	rs4684083	C	0.28	0.136	0.82	0.62	1.07	C	0.13	0.789	1.15	0.40	3.29	C	0.08	0.120	3.25	0.68	15.50
CD200R1L	3	rs4682429	A	0.43	0.977	1.00	0.78	1.27	A	0.30	0.305	1.51	0.68	3.36	A	0.07	0.582	0.74	0.26	2.15
IL4R	4	rs10024216	A	0.36	0.887	0.98	0.76	1.27	A	0.41	0.308	0.69	0.33	1.42	A	0.51	0.226	1.46	0.79	2.68
IL4R	4	rs10004195	A	0.23	0.260	0.85	0.64	1.13	A	0.43	0.600	1.22	0.58	2.53	A	0.45	0.342	1.36	0.72	2.57
SLC39A8	4	rs10014145	G	0.32	0.764	0.96	0.74	1.25	G	0.27	0.147	1.88	0.80	4.46	G	0.14	0.788	1.13	0.47	2.74
IL4	5	rs2243250	T	0.20	0.097 A 0.08 T	1.31	0.95	1.80	C	0.41	0.422	0.75	0.37	1.52	C	0.19	0.061 A 0.070 T	0.52	0.26	1.04
IL4	5	rs2070874	T	0.20	0.097 A 0.081 T	1.31	0.95	1.80	C	0.42	0.436	0.76	0.38	1.53	C	0.19	0.061 A 0.070 T	0.52	0.26	1.04
IL4	5	rs2243268	C	0.20	0.128	1.28	0.93	1.76	A	0.42	0.443	0.76	0.38	1.54	A	0.19	0.061	0.52	0.26	1.04
IL4	5	rs2243290	A	0.20	0.126	1.28	0.93	1.76	C	0.37	0.176	0.61	0.30	1.25	C	0.20	0.175	0.62	0.31	1.24

			Caucasians (n=519)						Admixed (n=64)						East Asian (n=90)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
IL4	5	rs12186803	A	0.19	0.155	1.27	0.91	1.75	G	0.35	**0.028 A 0.969 B 0.027 T**	0.45	0.22	0.92	G	0.21	0.085 A 0.070 T	0.55	0.28	1.09
ADRB2	5	rs877741	C	0.21	0.838	0.97	0.72	1.31	C	0.43	0.266	0.66	0.32	1.37	T	0.25	0.425	1.35	0.65	2.80
HLA	6	rs7192	T	0.37	0.823	1.03	0.80	1.33	T	0.45	0.545	1.24	0.61	2.51	T	0.27	0.891	0.95	0.49	1.86
TYRP1	9	rs1952692	C	0.32	0.300	0.87	0.67	1.13	C	0.17	0.394	0.68	0.28	1.66	C	0.06	0.655	1.38	0.33	5.71
TLR4	9	rs7849955	A	0.16	0.214	1.25	0.88	1.77	A	0.07	0.880	0.90	0.23	3.52	A	0.01	0.351	0.33	0.03	3.75
TLR4	9	rs1930713	A	0.22	0.207	0.83	0.62	1.11	A	0.12	0.342	0.62	0.23	1.68	A	0.05	0.883	1.12	0.26	4.82
TLR4	9	rs2245960	T	0.17	0.300	0.84	0.61	1.16	T	0.12	0.613	0.77	0.27	2.16	T	0.05	0.529	1.70	0.32	9.01
GATA3	10	rs10905349	A	0.23	0.197	1.22	0.90	1.64	A	0.40	0.368	1.39	0.68	2.88	G	0.48	0.761	1.10	0.60	2.00
IFNG	12	rs2069705	G	0.32	0.821	1.03	0.79	1.34	A	0.34	0.365	0.71	0.34	1.48	A	0.33	0.487	1.26	0.65	2.44
PHF11	13	rs7329078	T	0.40	0.396	1.12	0.87	1.43	T	0.22	0.318	0.66	0.30	1.49	T	0.23	0.326	0.71	0.36	1.41
LIG4	13	rs1805388	A	0.15	0.597	1.10	0.77	1.56	A	0.29	**0.042 A 1.0 B 0.057 T**	2.53	1.02	6.30	A	0.25	0.444	1.34	0.63	2.85
IRS2	13	rs2289046	C	0.35	0.530	1.09	0.84	1.40	C	0.37	0.855	0.94	0.46	1.92	T	0.47	1.000	1.00	0.55	1.82
PTGER2	14	rs708491	G	0.26	0.523	0.91	0.68	1.22	G	0.07	0.539	1.62	0.35	7.55	G	0.02	0.673	0.65	0.09	4.75
STAT5B	17	rs16967593	A	0.26	**0.001 A 0.047 B 0.004 G 0.001 T 0.003 D 0.023 R**	0.65	0.49	0.84	A	0.28	0.814	1.10	0.50	2.39	A	0.39	0.709	0.89	0.49	1.64
TGFb1	19	rs2241712	C	0.36	0.641	1.07	0.82	1.39	C	0.43	0.370	1.39	0.68	2.85	T	0.44	0.825	0.93	0.50	1.72

A1 – minor allele of SNP; OR – odds ratio for allelic trait model, L95/U95 – lower and upper range of 95% confidence interval for allelic trait model; NA – not applicable as odds ratio could not be computed since there were no infants with the major allele within the population group

[i] For SNPs that showed some evidence of association (nominal $P < 0.1$), several other tests based on a genotypic trait model were carried out to test for an association. The p-values for these tests are given in the table. Bonferroni adjusted *P* values are given for SNPs with nominal *P* value <0.05.

[ii]A – allelic trait model, B – Bonferroni adjusted, G – genotypic trait model, T – trend/additive trait model, D – dominant trait model, R – recessive trait model. Default model of analyses is the allelic trait model.

[iii] ORs and CIs shown are associated with the default model of analyses - allelic trait model

7.4.2 *HLA* variant, rs7192, is associated with increased risk of peanut allergy

Associations with specific foods – namely peanut (Table 7.3) and egg allergy (Table 7.4) were also tested. There was significant evidence for an association between rs7192 (*HLA*) and peanut allergy in Caucasian infants. The T allele was associated with an increased risk of food allergy ($P_{unadjusted}$=9.65 x 10^{-4}; OR=1.84, CI=1.28-2.65; $P_{adjusted}$=0.034). This SNP was however not associated with peanut allergy in the admixed and East Asian population groups.

There was also suggestive evidence that the *STAT5B* variant, rs16967593, associated with any food allergy, was also associated with peanut allergy in Caucasian infants ($P_{unadjusted}$=0.042; OR=0.64, 95% CI=0.42-0.99). However, this did not survive Bonferroni correction (Table 7.3). Another variant, rs231735 (*CTLA4*) had weak evidence of association with peanut allergy among Caucasian infants ($P_{unadjusted}$ =0.059; OR=1.42, 95% CI 0.99-2.05). When analysed under other models, rs231735 was associated with food allergy in the additive ($P_{unadjusted}$ =0.046) and dominant models ($P_{unadjusted}$ =0.038). None of the investigated SNPs showed evidence for association with peanut allergy in the admixed and East Asian infants after multiple testing adjustment under the allelic models ($P_{adjusted}$ > 0.05).

Similarly, for egg allergy, none of the investigated SNPs remained significantly associated in all three population groups after multiple testing (Table 7.4). However, when analysed under the various trait models, there was weak evidence of association between egg allergy and an interleukin 4 (*IL4*) variant, rs12186803 in the East Asian infants under the additive model ($P_{unadjusted}$ =0.056).

Table 7.3 Association of investigated SNPs with peanut allergy by parent's country of birth (non-atopic controls vs peanut allergics)

			Caucasians (n=521)						Admixed (n=60)						East Asian (n=80)					
Gene	**CHR**	**SNP**	**A1**	**MAF cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
ADAM17	2	rs10495562	T	0.44	0.389	0.85	0.59	1.23	C	0.15	0.748	0.82	0.25	2.70	C	0.06	0.942	1.06	0.21	5.37
ADAM17	2	rs1056204	C	0.34	0.273	1.24	0.84	1.82	C	0.12	0.768	1.23	0.31	4.92	C	0.03	0.317	3.79	0.23	62.18
KCNS3	2	rs4368333	C	0.41	0.431	0.86	0.60	1.25	A	0.15	0.153	0.44	0.14	1.39	A	0.12	0.08 A 0.106 T	3.23	0.82	12.75
SOCS5	2	rs6737848	G	0.13	0.035 A 1 B 0.031 T	1.80	1.04	3.12	G	0.31	0.889	1.07	0.42	2.76	G	0.44	0.754	1.13	0.53	2.43
EDAR	2	rs6749207	C	0.11	0.186	0.68	0.39	1.21	T	0.21	0.008 A 0.278 B **0.05 T**	0.25	0.09	0.73	T	0.16	0.158	0.48	0.17	1.35
DPP10	2	rs4353658	A	0.41	0.758	1.06	0.73	1.53	A	0.31	0.754	0.86	0.34	2.20	A	0.18	0.323	1.68	0.59	4.78
DPP10	2	rs7579207	A	0.37	0.162	1.31	0.90	1.91	A	0.15	0.951	1.04	0.31	3.47	A	0.06	0.298	2.56	0.41	15.99
CTLA4	2	rs231735	G	0.53	0.059 A 1 B 0.104 G **0.046 T** **0.038 D** 0.293 R	1.42	0.99	2.05	G	0.46	0.303	1.58	0.66	3.82	G	0.21	0.532	0.75	0.30	1.88
CTLA4	2	rs231804	C	0.49	**0.034 A** 1 B **0.048 G** **0.026 T** **0.014 D** 0.359 R	1.48	1.03	2.13	C	0.46	0.117	2.02	0.83	4.91	C	0.19	0.487	0.71	0.27	1.88
CTLA4	2	rs11571291	C	0.50	**0.025 A** 0.859 B **0.019 G** **0.020 T** **0.005 D** 0.494 R	1.52	1.05	2.18	C	0.42	0.173	1.86	0.76	4.58	C	0.21	0.594	0.78	0.31	1.96

			Caucasians (n=521)						Admixed (n=60)						East Asian (n=80)					
Gene	**CHR**	**SNP**	**A1**	**MAF cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
IKZF2	2	rs12619285	G	0.25	0.956	0.99	0.65	1.51	G	0.38	0.348	0.65	0.27	1.59	A	0.44	0.628	1.21	0.56	2.60
CHL1	3	rs4684083	C	0.20	**0.005 A** 0.188 B **0.004 T**	0.54	0.34	0.84	C	0.12	0.761	0.81	0.21	3.10	C	0.06	0.732	1.33	0.26	6.94
CD200R1L	3	rs4682429	A	0.46	0.498	1.13	0.79	1.63	A	0.35	0.248	1.73	0.68	4.43	A	0.06	0.590	0.65	0.14	3.10
IL4R	4	rs10024216	A	0.30	0.144	0.75	0.50	1.11	A	0.23	**0.013 A** 0.450 B **0.012 T**	0.27	0.09	0.79	A	0.53	0.516	1.30	0.59	2.83
IL4R	4	rs10004195	A	0.18	**0.027 A** 0.946 B **0.028 T**	0.59	0.37	0.95	A	0.38	0.676	0.82	0.33	2.07	A	0.41	0.947	0.97	0.45	2.13
SLC39A8	4	rs10014145	G	0.30	0.555	0.89	0.59	1.32	G	0.35	**0.024 A** 0.783 B **0.022 T**	3.03	1.13	8.12	G	0.18	0.540	1.37	0.50	3.81
IL4	5	rs2243250	T	0.19	0.451	1.19	0.75	1.90	C	0.38	0.508	0.74	0.31	1.80	C	0.15	0.190	0.51	0.18	1.42
IL4	5	rs2070874	T	0.19	0.566	1.15	0.72	1.83	C	0.38	0.508	0.74	0.31	1.80	C	0.15	0.190	0.51	0.18	1.42
IL4	5	rs2243268	C	0.19	0.559	1.15	0.72	1.84	A	0.38	0.514	0.74	0.31	1.81	A	0.15	0.190	0.51	0.18	1.42
IL4	5	rs2243290	A	0.19	0.559	1.15	0.72	1.84	C	0.31	0.172	0.53	0.21	1.33	C	0.18	0.346	0.63	0.24	1.66
IL4	5	rs12186803	A	0.18	0.509	1.17	0.73	1.89	G	0.31	0.066 A 0.066 T	0.43	0.17	1.08	G	0.21	0.532	0.75	0.30	1.88
ADRB2	5	rs877741	C	0.24	0.433	1.19	0.77	1.84	C	0.29	**0.045 A** 1 B **0.035 T**	0.38	0.14	1.00	T	0.29	0.745	1.15	0.50	2.66
HLA	6	rs7192	T	0.50	**0.000965 A 0.034 B 0.002 G 9.72x10^{-4} T 0.023 D**	1.84	1.28	2.65	T	0.50	0.240	1.69	0.70	4.04	T	0.29	0.637	1.22	0.53	2.83

			Caucasians (n=521)						Admixed (n=60)						East Asian (n=80)					
Gene	**CHR**	**SNP**	**A1**	**MAF cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
					$9.17x10^{-4}$ R															
TYRP1	9	rs1952692	C	0.31	0.350	0.83	0.56	1.23	C	0.15	0.549	0.70	0.21	2.27	C	0.00	NA	0.00	0.00	NA
TLR4	9	rs7849955	A	0.19	0.138	1.43	0.89	2.30	A	0.08	0.966	1.04	0.20	5.31	A	0.03	0.569	2.00	0.18	22.77
TLR4	9	rs1930713	A	0.25	0.702	1.09	0.71	1.66	A	0.12	0.664	0.75	0.20	2.82	A	0.06	0.461	1.91	0.33	10.88
TLR4	9	rs2245960	T	0.17	0.805	0.94	0.58	1.52	T	0.12	0.867	0.89	0.23	3.43	T	0.06	0.298	2.56	0.41	15.99
GATA3	10	rs10905349	A	0.26	0.120	1.39	0.92	2.11	A	0.46	0.117	2.02	0.83	4.91	G	0.41	0.453	0.75	0.35	1.61
IFNG	12	rs2069705	G	0.36	0.405	1.18	0.80	1.72	A	0.29	0.145	0.49	0.19	1.30	A	0.22	0.221	0.57	0.23	1.42
PHF11	13	rs7329078	T	0.37	0.454	0.87	0.59	1.26	T	0.23	0.568	0.74	0.27	2.06	T	0.22	0.470	0.71	0.28	1.80
LIG4	13	rs1805388	A	0.15	0.722	1.10	0.65	1.86	A	0.13	0.364	0.55	0.15	2.04	A	0.29	0.167	1.85	0.77	4.43
IRS2	13	rs2289046	C	0.37	0.298	1.22	0.84	1.78	C	0.50	0.330	1.54	0.64	3.69	T	0.47	0.977	1.01	0.47	2.16
PTGER2	14	rs708491	G	0.26	0.821	0.95	0.62	1.47	G	0.17	0.034 A 1 B 0.028 T	4.40	1.01	19.11	G	0.03	0.864	1.22	0.12	12.14
STAT5B	17	rs16967593	A	0.23	**0.042 A** 1 B **0.037 T**	0.64	0.42	0.99	A	0.31	0.756	1.16	0.45	3.00	A	0.26	0.134	0.53	0.23	1.23
TGFb1	19	rs2241712	C	0.28	0.109	0.72	0.48	1.08	C	0.54	0.108	2.09	0.84	5.16	T	0.47	0.769	1.13	0.51	2.47

A1 – minor allele of SNP; OR – odds ratio for allelic trait model, L95/U95 – lower and upper range of 95% confidence interval for allelic trait model; NA – not applicable as odds ratio could not be computed since there were no infants with the major allele within the population group

[i] For SNPs that showed some evidence of association (nominal $P < 0.1$), several other tests based on a genotypic trait model were carried out to test for an association. The p-values for these tests are given in the table. Bonferroni adjusted *P* values are given for SNPs with nominal *P* value <0.05.

[ii] A – allelic trait model, B – Bonferroni adjusted, G – genotypic trait model, T – trend/additive trait model, D – dominant trait model, R – recessive trait model. Default model of analyses is the allelic trait model.

[iii] ORs and CIs shown are associated with the default model of analyses - allelic trait model.

Table 7.4 Association of investigated SNPs with egg allergy by parent's country of birth (non-atopic controls vs egg allergics)

			Caucasians (n=547)						Admixed (n=67)						East Asian (n=94)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
ADAM17	2	rs10495562	T	0.50	0.250	1.15	0.90	1.47	C	0.22	0.498	1.35	0.57	3.19	C	0.03	0.363	0.51	0.12	2.21
ADAM17	2	rs1056204	C	0.31	0.916	1.01	0.78	1.32	C	0.13	0.295	1.84	0.58	5.80	C	0.00	NA	NA	NA	NA
KCNS3	2	rs4368333	C	0.43	0.405	0.90	0.71	1.15	A	0.22	0.353	0.68	0.31	1.53	A	0.03	0.076 A 0.095 T	0.31	0.08	1.20
SOCS5	2	rs6737848	G	0.09	0.869	0.97	0.63	1.47	G	0.33	0.298	1.51	0.69	3.28	G	0.45	0.928	0.97	0.54	1.74
EDAR	2	rs6749207	C	0.16	0.891	1.02	0.73	1.43	T	0.33	**0.035 A** 1 B **0.023 T**	0.45	0.22	0.95	T	0.27	0.951	1.02	0.53	1.96
DPP10	2	rs4353658	A	0.41	0.266	1.15	0.90	1.48	A	0.37	0.696	1.16	0.56	2.38	A	0.15	0.665	1.20	0.52	2.78
DPP10	2	rs7579207	A	0.31	0.983	1.00	0.77	1.30	A	0.17	0.949	1.03	0.41	2.61	A	0.04	0.501	1.79	0.32	10.03
CTLA4	2	rs231735	G	0.46	0.625	0.94	0.74	1.20	G	0.39	0.871	1.06	0.52	2.16	G	0.17	0.086 A 0.119 T	0.54	0.27	1.10
CTLA4	2	rs231804	C	0.42	0.976	1.00	0.78	1.28	C	0.35	0.747	1.13	0.54	2.34	C	0.16	**0.057 A** 0.079 T	0.50	0.25	1.03
CTLA4	2	rs11571291	C	0.43	0.593	1.07	0.84	1.37	C	0.31	0.835	0.92	0.44	1.96	C	0.17	0.103 A 0.140 T	0.56	0.28	1.13
IKZF2	2	rs12619285	G	0.23	0.155	0.81	0.61	1.08	G	0.44	0.943	0.97	0.48	1.97	A	0.39	0.282	0.73	0.41	1.30
CHL1	3	rs4684083	C	0.27	0.054 A 1 B 0.133 G **0.045 T** 0.06 D 0.235 R	0.77	0.59	1.00	C	0.13	0.896	0.93	0.34	2.58	C	0.08	0.075 A 0.067 T	3.82	0.79	18.51
CD200R1L	3	rs4682429	A	0.43	0.999	1.00	0.78	1.28	A	0.31	0.322	1.48	0.68	3.19	A	0.08	0.595	0.76	0.28	2.07
IL4R	4	rs10024216	A	0.36	0.674	1.06	0.82	1.36	A	0.44	0.587	0.82	0.40	1.69	A	0.52	0.227	1.44	0.80	2.58
IL4R	4	rs10004195	A	0.24	0.482	0.90	0.68	1.20	A	0.44	0.657	1.18	0.57	2.44	A	0.45	0.452	1.26	0.69	2.32
SLC39A8	4	rs10014145	G	0.32	0.978	1.00	0.77	1.30	G	0.22	0.813	1.11	0.48	2.58	G	0.12	0.917	0.95	0.40	2.29

			Caucasians (n=547)						Admixed (n=67)						East Asian (n=94)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
IL4	5	rs2243250	T	0.20	0.244	1.20	0.88	1.64	C	0.42	0.558	0.81	0.40	1.64	C	0.18	0.09 A 0.1 T	0.55	0.28	1.10
IL4	5	rs2070874	T	0.20	0.255	1.20	0.88	1.64	C	0.43	0.576	0.82	0.41	1.64	C	0.18	0.09 A 0.1 T	0.55	0.28	1.10
IL4	5	rs2243268	C	0.19	0.297	1.18	0.86	1.62	A	0.43	0.583	0.82	0.41	1.65	A	0.18	0.09 A 0.1 T	0.55	0.28	1.10
IL4	5	rs2243290	A	0.20	0.315	1.17	0.86	1.60	C	0.39	0.325	0.70	0.35	1.42	C	0.19	0.128	0.59	0.30	1.17
IL4	5	rs12186803	A	0.18	0.397	1.15	0.83	1.58	G	0.37	0.078 A 0.075 T	0.53	0.26	1.08	G	0.20	0.051 A 0.056 T	0.52	0.27	1.01
ADRB2	5	rs877741	C	0.20	0.369	0.87	0.64	1.18	C	0.43	0.412	0.74	0.36	1.52	T	0.22	0.964	0.98	0.49	1.96
HLA	6	rs7192	T	0.36	0.406	0.90	0.70	1.16	T	0.44	0.590	1.21	0.60	2.45	T	0.25	0.300	0.71	0.38	1.35
TYRP1	9	rs1952692	C	0.32	0.496	0.91	0.71	1.18	C	0.17	0.707	0.84	0.34	2.09	C	0.06	0.409	1.80	0.44	7.45
TLR4	9	rs7849955	A	0.15	0.420	1.15	0.82	1.63	A	0.07	0.984	0.99	0.26	3.68	A	0.00	NA	NA	NA	NA
TLR4	9	rs1930713	A	0.22	0.457	0.90	0.67	1.20	A	0.13	0.600	0.77	0.28	2.07	A	0.05	0.884	1.11	0.29	4.25
TLR4	9	rs2245960	T	0.17	0.657	0.93	0.68	1.28	T	0.13	0.937	1.04	0.37	2.93	T	0.05	0.590	1.49	0.35	6.43
GATA3	10	rs10905349	A	0.22	0.247	1.19	0.89	1.61	A	0.35	0.785	0.90	0.44	1.86	G	0.48	0.727	1.11	0.62	1.97
IFNG	12	rs2069705	G	0.33	0.591	1.08	0.83	1.40	A	0.37	0.465	0.77	0.38	1.57	A	0.33	0.372	1.33	0.71	2.51
PHF11	13	rs7329078	T	0.40	0.659	1.06	0.82	1.36	T	0.20	0.239	0.61	0.27	1.39	T	0.22	0.269	0.69	0.35	1.34
LIG4	13	rs1805388	A	0.15	0.784	1.05	0.74	1.49	A	0.32	**0.015 A** 0.541 B **0.027 T**	2.87	1.20	6.85	A	0.27	0.152	1.71	0.82	3.57
IRS2	13	rs2289046	C	0.34	0.559	1.08	0.83	1.40	C	0.33	0.434	0.75	0.36	1.54	T	0.48	0.784	0.92	0.52	1.64
PTGER2	14	rs708491	G	0.26	0.432	0.89	0.67	1.19	G	0.08	0.731	1.27	0.32	4.97	G	0.01	0.252	0.29	0.03	2.79
STAT5B	17	rs16967593	A	0.27	**0.019 A** 0.678 B 0.056 G **0.016 T** **0.031 D** 0.104 R	0.73	0.56	0.95	A	0.28	0.972	1.01	0.47	2.19	A	0.40	0.659	0.88	0.49	1.57

			Caucasians (n=547)						Admixed (n=67)						East Asian (n=94)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
TGFb1	19	rs2241712	C	0.37	0.203	1.19	0.91	1.54	C	0.38	0.750	0.89	0.44	1.82	T	0.44	0.815	0.93	0.52	1.68

A1 – minor allele of SNP; OR – odds ratio for allelic trait model, L95/U95 – lower and upper range of 95% confidence interval for allelic trait model; NA – not applicable as odds ratio could not be computed since there were no infants with the major allele within the population group

[i] For SNPs that showed some evidence of association (nominal $P < 0.1$), several other tests based on a genotypic trait model were carried out to test for an association. The p-values for these tests are given in the table. Bonferroni adjusted *P* values are given for SNPs with nominal *P* value <0.05.

[ii] A – allelic trait model, B – Bonferroni adjusted, G – genotypic trait model, T – trend/additive trait model, D – dominant trait model, R – recessive trait model. Default model of analyses is the allelic trait model.

[iii] ORs and CIs shown are associated with the default model of analyses - allelic trait model.

7.4.3 *STAT5B* variant, rs16967593, is associated with reduced risk of sensitisation to any food

The second set of analyses compared the non-sensitised non-allergic controls with food sensitised tolerant infants. This analysis assessed the associations with food sensitisation, which is a biological precursor to subsequent development of food allergy.

Interestingly, the same *STAT5B* variant associated with any food allergy, rs16967593, was also associated with sensitisation to any food among Caucasian infants (Table 7.5). Those carrying the A allele had a reduced risk of developing any food sensitisation ($P_{un-adjusted}$ =3.41 x 10^{-4}; OR= 0.53, 95% CI 0.37-0.75; $P_{adjusted}$=0.012). This variant was also associated with a reduced risk of egg sensitisation (P=0.001; OR=0.39, 95% CI 0.21-0.70; $P_{adjusted}$=0.042) in Caucasian infants. In the admixed ($P_{unadjusted}$ =0.033) and East Asian ($P_{unadjusted}$ =0.017) populations, an association between rs16967593 and egg sensitisation was only found under the additive model (Table 7.6).

While there is suggestive evidence that several variants of *IL4*, are associated with any food sensitisation (Table 7.5) and peanut sensitisation (Table 7.7) in the Caucasian infants, none of these survived Bonferroni correction under allelic trait model. For variant rs12186803, the A allele was associated with increased risk of any food sensitisation in Caucasian infants whereas the G allele was associated with a reduced risk of any food sensitisation in the admixed population ($P_{unadjusted}$ =0.026; OR=0.36, 95% CI 0.14-0.90). Although these did not survive multiple testing adjustment ($P_{adjusted}$=0.571 in Caucasian; $P_{adjusted}$=0.897 in admixed), rs12186803 was also associated with any food sensitisation in both Caucasian ($P_{unadjusted}$ =0.014) and admixed ($P_{unadjusted}$ =0.021) population group under the additive model (Table 7.5).

Three SNPs in *CTLA4* (rs231735, rs231804, rs11571291) showed weak evidence for an increased risk with egg sensitisation in East Asian infants. However, none of the investigated SNPs remained significant after correction (Table 7.5).

Table 7.5 Association of investigated SNPs with 'any food sensitisation' by parent's country of birth (non-atopic controls vs food sensitised tolerant)

			Caucasians (n=395)						Admixed (n=49)						East Asian (n=58)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
ADAM17	2	rs10495562	T	0.45	0.509	0.90	0.66	1.23	C	0.17	0.773	0.85	0.27	2.63	C	0.00	NA	NA	NA	NA
ADAM17	2	rs1056204	C	0.34	0.281	1.20	0.86	1.66	C	0.03	0.331	0.36	0.04	3.10	C	0.00	NA	NA	NA	NA
KCNS3	2	rs4368333	C	0.44	0.622	0.93	0.68	1.26	A	0.20	0.233	0.54	0.19	1.51	A	0.07	0.979	0.98	0.22	4.32
SOCS5	2	rs6737848	G	0.10	0.543	1.18	0.70	1.99	G	0.29	0.660	1.25	0.46	3.38	G	0.52	0.210	1.63	0.76	3.51
EDAR	2	rs6749207	C	0.15	0.890	0.97	0.63	1.49	T	0.29	**0.029 A** 1 B **0.016 T**	0.36	0.14	0.92	T	0.25	1.000	1.00	0.42	2.38
DPP10	2	rs4353658	A	0.37	0.945	1.01	0.74	1.38	A	0.40	0.557	1.30	0.54	3.17	A	0.16	0.813	1.14	0.40	3.24
DPP10	2	rs7579207	A	0.33	0.608	1.09	0.79	1.51	A	0.13	0.719	0.80	0.23	2.74	A	0.02	0.723	1.65	0.10	27.09
CTLA4	2	rs231735	G	0.44	0.431	0.88	0.65	1.20	G	0.30	0.433	0.69	0.28	1.74	G	0.24	0.955	0.97	0.40	2.39
CTLA4	2	rs231804	C	0.40	0.715	0.94	0.69	1.29	C	0.27	0.574	0.76	0.29	1.98	C	0.24	0.955	0.97	0.40	2.39
CTLA4	2	rs11571291	C	0.39	0.669	0.93	0.68	1.28	C	0.27	0.574	0.76	0.29	1.98	C	0.25	0.865	1.08	0.45	2.58
IKZF2	2	rs12619285	G	0.27	0.802	1.05	0.74	1.48	G	0.43	0.740	0.86	0.36	2.06	A	0.39	0.639	0.83	0.39	1.79
CHL1	3	rs4684083	C	0.29	0.431	0.87	0.63	1.22	C	0.10	0.799	0.83	0.21	3.39	C	0.11	0.06 A 0.051 T	4.49	0.83	24.22
CD200R1L	3	rs4682429	A	0.44	0.757	1.05	0.77	1.43	A	0.20	0.819	0.88	0.31	2.56	A	0.07	0.589	0.68	0.17	2.78
IL4R	4	rs10024216	A	0.34	0.511	0.90	0.65	1.24	A	0.43	0.524	0.75	0.31	1.82	A	0.48	0.523	1.28	0.60	2.72
IL4R	4	rs10004195	A	0.20	0.066 A 0.073 T	0.71	0.49	1.02	A	0.42	0.695	1.20	0.48	3.03	A	0.48	0.275	1.54	0.71	3.34
SLC39A8	4	rs10014145	G	0.31	0.637	0.92	0.66	1.29	G	0.25	0.314	1.73	0.59	5.04	G	0.14	0.860	1.11	0.36	3.35

			Caucasians (n=395)						Admixed (n=49)						East Asian (n=58)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
IL4	5	rs2243250	T	0.23	**0.017 A** 0.610 B **0.050 G** **0.015 T** **0.023 D** 0.167 R	1.58	1.08	2.30	C	0.43	0.613	0.80	0.33	1.93	C	0.20	0.233	0.58	0.24	1.42
IL4	5	rs2070874	T	0.23	**0.014 A** 0.507 B **0.043 G** **0.012 T** **0.019 D** 0.167 R	1.60	1.10	2.34	C	0.43	0.635	0.81	0.34	1.93	C	0.20	0.233	0.58	0.24	1.42
IL4	5	rs2243268	C	0.23	**0.016 A** 0.573 B **0.013 T**	1.59	1.09	2.31	A	0.43	0.639	0.81	0.34	1.94	A	0.20	0.233	0.58	0.24	1.42
IL4	5	rs2243290	A	0.23	**0.018 A** 0.629 B **0.051 G** **0.015 T** **0.023 D** 0.163 R	1.57	1.08	2.30	C	0.37	0.277	0.61	0.25	1.48	C	0.20	0.298	0.62	0.26	1.52
IL4	5	rs12186803	A	0.22	**0.016 A** 0.571 B **0.014 T**	1.60	1.09	2.34	G	0.30	**0.026 A** 0.897 B **0.021 T**	0.36	0.14	0.90	G	0.25	0.372	0.68	0.29	1.59
ADRB2	5	rs877741	C	0.20	0.650	0.92	0.63	1.34	C	0.53	1.000	1.00	0.42	2.41	T	0.23	0.672	1.22	0.49	3.04
HLA	6	rs7192	T	0.37	0.981	1.00	0.73	1.38	T	0.39	0.970	0.98	0.40	2.42	T	0.36	0.332	1.49	0.67	3.31
TYRP1	9	rs1952692	C	0.38	0.333	1.17	0.85	1.61	C	0.13	0.284	0.52	0.16	1.74	C	0.05	0.922	1.10	0.18	6.83
TLR4	9	rs7849955	A	0.13	0.823	1.05	0.67	1.65	A	0.03	0.444	0.43	0.05	3.89	A	0.00	NA	NA	NA	NA
TLR4	9	rs1930713	A	0.22	0.291	0.82	0.57	1.18	A	0.10	0.333	0.52	0.14	1.99	A	0.07	0.532	1.68	0.32	8.73
TLR4	9	rs2245960	T	0.17	0.526	0.88	0.59	1.31	T	0.07	0.263	0.41	0.09	2.02	T	0.07	0.299	2.56	0.41	15.97
GATA3	10	rs10905349	A	0.20	0.953	1.01	0.69	1.48	A	0.40	0.464	1.39	0.57	3.39	G	0.48	0.843	1.08	0.51	2.29

			Caucasians (n=395)						Admixed (n=49)						East Asian (n=58)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
IFNG	12	rs2069705	G	0.33	0.671	1.07	0.77	1.49	A	0.37	0.594	0.79	0.32	1.91	A	0.23	0.547	0.76	0.32	1.83
PHF11	13	rs7329078	T	0.40	0.577	1.09	0.80	1.50	T	0.18	0.241	0.52	0.17	1.57	T	0.20	0.298	0.62	0.26	1.52
LIG4	13	rs1805388	A	0.16	0.424	1.19	0.78	1.83	A	0.21	0.346	1.73	0.55	5.42	A	0.26	0.479	1.39	0.56	3.49
IRS2	13	rs2289046	C	0.26	0.056 A 0.057 G 0.057 T **0.019 D** 0.783 R	0.72	0.51	1.01	C	0.47	0.434	1.41	0.59	3.37	T	0.43	0.672	0.85	0.40	1.81
PTGER2	14	rs708491	G	0.23	0.200	0.78	0.54	1.14	G	0.07	0.608	1.62	0.25	10.24	G	0.00	NA	NA	NA	NA
STAT5B	17	rs16967593	A	0.22	**0.00034 A** **0.012 B** **4.13x10^{-4} G** **3.25x10^{-4} T** **7.92x10^{-5} D** 0.200 R	0.53	0.37	0.75	A	0.37	0.308	1.61	0.64	4.03	A	0.45	0.710	1.16	0.54	2.49
TGFb1	19	rs2241712	C	0.36	0.671	1.07	0.77	1.49	C	0.50	0.171	1.83	0.77	4.38	T	0.43	0.768	0.89	0.41	1.93

A1 – minor allele of SNP; OR – odds ratio for allelic trait model, L95/U95 – lower and upper range of 95% confidence interval for allelic trait model; NA – not applicable as odds ratio could not be computed since there were no infants with the major allele within the population group

[i] For SNPs that showed some evidence of association (nominal $P < 0.1$), several other tests based on a genotypic trait model were carried out to test for an association. The p-values for these tests are given in the table. Bonferroni adjusted *P* values are given for SNPs with nominal *P* value <0.05.

[ii] A – allelic trait model, B – Bonferroni adjusted, G – genotypic trait model, T – trend/additive trait model, D – dominant trait model, R – recessive trait model. Default model of analyses is the allelic trait model.

[iii] ORs and CIs shown are associated with the default model of analyses - allelic trait model.

Table 7.6 Association of investigated SNPs with egg sensitisation by parent's country of birth (non-atopic controls vs egg sensitised tolerant)

			Caucasians (n=374)						Admixed (n=43)						East Asian (n=48)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
ADAM17	2	rs10495562	T	0.44	0.723	0.92	0.59	1.45	C	0.00	NA	0.00	0.00	NA	C	0.00	NA	NA	NA	NA
ADAM17	2	rs1056204	C	0.35	0.397	1.23	0.76	1.97	C	0.00	NA	0.00	0.00	NA	C	0.00	NA	NA	NA	NA
KCNS3	2	rs4368333	C	0.44	0.870	0.96	0.61	1.51	A	0.17	0.503	0.48	0.05	4.32	A	0.13	0.752	1.43	0.16	13.12
SOCS5	2	rs6737848	G	0.06	0.278	0.60	0.23	1.53	G	0.33	0.625	1.55	0.26	9.16	G	0.13	0.071 A 0.098 T	0.17	0.02	1.45
EDAR	2	rs6749207	C	0.11	0.229	0.64	0.31	1.33	T	0.00	NA	0.00	0.00	NA	T	0.13	0.394	0.40	0.05	3.46
DPP10	2	rs4353658	A	0.49	**0.036 A** 1 B 0.060 G **0.034 T** 0.182 D **0.023 R**	1.61	1.03	2.54	A	0.33	0.983	0.98	0.17	5.70	A	0.13	0.981	0.97	0.11	8.69
DPP10	2	rs7579207	A	0.36	0.387	1.23	0.77	1.97	A	0.00	NA	0.00	0.00	NA	A	0.00	NA	0.00	0.00	NA
CTLA4	2	rs231735	G	0.37	0.07 A 0.053 T	0.65	0.41	1.04	G	0.33	0.839	0.83	0.14	4.83	G	0.63	**0.043 A** 1 B **0.048 T**	4.31	0.95	19.43
CTLA4	2	rs231804	C	0.36	0.254	0.76	0.47	1.22	C	0.33	0.967	1.04	0.18	6.04	C	0.67	**0.046 A** 1 B 0.054 T	5.17	0.89	30.08
CTLA4	2	rs11571291	C	0.34	0.150	0.71	0.44	1.14	C	0.33	0.967	1.04	0.18	6.04	C	0.63	**0.038 A** 1 B **0.044 T**	4.44	0.99	20.04
IKZF2	2	rs12619285	G	0.24	0.582	0.86	0.51	1.46	G	0.67	0.302	2.46	0.42	14.21	A	0.25	0.240	0.38	0.07	2.00
CHL1	3	rs4684083	C	0.33	0.997	1.00	0.62	1.61	C	0.00	NA	0.00	0.00	NA	C	0.13	0.111	6.14	0.49	76.43
CD200R1L	3	rs4682429	A	0.48	0.426	1.20	0.76	1.90	A	0.00	NA	0.00	0.00	NA	A	0.00	NA	0.00	0.00	NA
IL4R	4	rs10024216	A	0.33	0.740	0.92	0.57	1.49	A	0.33	0.467	0.53	0.09	3.04	A	0.63	0.293	2.19	0.49	9.75
IL4R	4	rs10004195	A	0.18	0.126	0.63	0.35	1.14	A	0.50	0.683	1.52	0.20	11.34	A	0.50	0.564	1.53	0.36	6.53

			Caucasians (n=374)						Admixed (n=43)						East Asian (n=48)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
SLC39A8	4	rs10014145	G	0.32	0.919	1.03	0.63	1.67	G	0.17	0.821	0.78	0.08	7.11	G	0.50	**0.005 A** 0.181 B **0.009 T**	7.00	1.53	32.11
IL4	5	rs2243250	T	0.23	0.162	1.47	0.86	2.52	C	0.33	0.502	0.55	0.10	3.19	C	0.13	0.332	0.36	0.04	3.08
IL4	5	rs2070874	T	0.23	0.151	1.48	0.86	2.54	C	0.33	0.502	0.55	0.10	3.19	C	0.13	0.332	0.36	0.04	3.08
IL4	5	rs2243268	C	0.23	0.151	1.48	0.86	2.54	A	0.33	0.505	0.55	0.10	3.20	A	0.13	0.332	0.36	0.04	3.08
IL4	5	rs2243290	A	0.24	0.142	1.50	0.87	2.57	C	0.17	0.143	0.22	0.02	1.98	C	0.13	0.332	0.36	0.04	3.08
IL4	5	rs12186803	A	0.22	0.179	1.46	0.84	2.52	G	0.17	0.09 A 0.079 T	0.18	0.02	1.62	G	0.13	0.240	0.30	0.03	2.52
ADRB2	5	rs877741	C	0.21	0.828	0.94	0.54	1.63	C	0.83	0.117	5.00	0.56	45.02	T	0.25	0.884	1.13	0.21	6.06
HLA	6	rs7192	T	0.38	0.961	0.99	0.62	1.58	T	0.33	0.757	0.76	0.13	4.39	T	0.25	0.690	0.71	0.14	3.76
TYRP1	9	rs1952692	C	0.42	0.190	1.36	0.86	2.16	C	0.17	0.878	0.84	0.09	7.73	C	0.13	0.227	3.95	0.36	43.18
TLR4	9	rs7849955	A	0.14	0.897	1.04	0.55	2.00	A	0.00	NA	NA	NA	NA	A	0.00	NA	NA	NA	NA
TLR4	9	rs1930713	A	0.22	0.671	0.89	0.52	1.53	A	0.00	NA	NA	NA	NA	A	0.00	NA	NA	NA	NA
TLR4	9	rs2245960	T	0.17	0.838	0.94	0.52	1.70	T	0.00	NA	NA	NA	NA	T	0.00	0.596	NA	NA	NA
GATA3	10	rs10905349	A	0.21	0.726	1.10	0.63	1.92	A	0.17	0.305	0.33	0.04	2.99	G	0.38	0.665	0.72	0.16	3.20
IFNG	12	rs2069705	G	0.38	0.240	1.33	0.83	2.14	A	0.50	0.755	1.30	0.25	6.88	A	0.13	0.362	0.38	0.04	3.26
PHF11	13	rs7329078	T	0.37	0.701	0.91	0.57	1.46	T	0.17	0.503	0.48	0.05	4.32	T	0.25	0.808	0.81	0.15	4.31
LIG4	13	rs1805388	A	0.13	0.724	0.89	0.45	1.73	A	0.00	NA	NA	NA	NA	A	0.13	0.720	0.67	0.08	5.92
IRS2	13	rs2289046	C	0.24	0.137	0.68	0.40	1.14	C	0.83	**0.039 A** 1 B **0.039 T**	7.50	0.84	67.22	T	0.25	0.175	0.33	0.06	1.74
PTGER2	14	rs708491	G	0.22	0.227	0.70	0.39	1.25	G	0.00	NA	NA	NA	NA	G	0.00	NA	NA	NA	NA

			Caucasians (n=374)						Admixed (n=43)						East Asian (n=48)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
STAT5B	17	rs16967593	A	0.16	**0.001 A** **0.042 B** **0.0009 T**	0.39	0.21	0.70	A	0.67	**0.044 A** 1 B **0.033 T**	5.27	0.90	30.86	A	0.00	NA	NA	NA	NA
TGFb1	19	rs2241712	C	0.39	0.241	1.34	0.82	2.19	C	0.67	0.225	2.85	0.49	16.47	T	0.67	0.312	2.41	0.42	13.87

A1 – minor allele of SNP; OR – odds ratio for allelic trait model, L95/U95 – lower and upper range of 95% confidence interval for allelic trait model; NA – not applicable as odds ratio could not be computed since there were no infants with the major allele within the population group

[i] For SNPs that showed some evidence of association (nominal $P < 0.1$), several other tests based on a genotypic trait model were carried out to test for an association. The p-values for these tests are given in the table. Bonferroni adjusted *P* values are given for SNPs with nominal *P* value <0.05.

[ii] A – allelic trait model, B – Bonferroni adjusted, G – genotypic trait model, T – trend/additive trait model, D – dominant trait model, R – recessive trait model. Default model of analyses is the allelic trait model.

[iii] ORs and CIs shown are associated with the default model of analyses - allelic trait model.

Table 7.7 Association of investigated SNPs with peanut sensitisation by parent's country of birth (non-atopic controls vs peanut sensitised tolerant)

			Caucasians (n=526)						Admixed (n=58)						East Asian (n=79)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
ADAM17	2	rs10495562	T	0.44	0.426	0.87	0.61	1.23	C	0.18	0.992	1.01	0.30	3.36	C	0.00	NA	NA	NA	NA
ADAM17	2	rs1056204	C	0.35	0.221	1.26	0.87	1.83	C	0.00	0.131	0.00	0.00	nan	C	0.00	NA	NA	NA	NA
KCNS3	2	rs4368333	C	0.41	0.407	0.86	0.60	1.23	A	0.27	0.847	0.90	0.32	2.55	A	0.06	0.575	1.61	0.30	8.73
SOCS5	2	rs6737848	G	0.10	0.471	1.25	0.68	2.28	G	0.30	0.954	1.03	0.36	2.97	G	0.60	0.062 A 0.073 T	2.15	0.95	4.84
EDAR	2	rs6749207	C	0.19	0.368	1.24	0.78	1.97	T	0.40	0.369	0.64	0.24	1.71	T	0.28	0.969	1.02	0.43	2.41
DPP10	2	rs4353658	A	0.34	0.199	0.79	0.54	1.14	A	0.41	0.544	1.34	0.52	3.47	A	0.19	0.260	1.81	0.64	5.17
DPP10	2	rs7579207	A	0.31	0.862	0.97	0.66	1.42	A	0.18	0.701	1.27	0.37	4.32	A	0.03	0.811	1.32	0.13	13.15
CTLA4	2	rs231735	G	0.49	0.331	1.19	0.84	1.69	G	0.36	0.912	1.06	0.40	2.78	G	0.10	0.064 A 0.074 T	0.32	0.09	1.13
CTLA4	2	rs231804	C	0.43	0.389	1.17	0.82	1.69	C	0.32	0.852	1.10	0.40	2.99	C	0.13	0.142	0.44	0.14	1.35
CTLA4	2	rs11571291	C	0.44	0.392	1.17	0.82	1.68	C	0.32	0.741	1.19	0.43	3.24	C	0.13	0.131	0.43	0.14	1.32
IKZF2	2	rs12619285	G	0.28	0.562	1.13	0.75	1.69	G	0.41	0.502	0.72	0.28	1.86	A	0.41	0.909	1.05	0.47	2.31
CHL1	3	rs4684083	C	0.29	0.496	0.87	0.59	1.29	C	0.14	0.981	0.98	0.25	3.80	C	0.09	0.315	2.07	0.49	8.77
CD200R1L	3	rs4682429	A	0.40	0.517	0.89	0.62	1.27	A	0.27	0.703	1.23	0.43	3.52	A	0.09	0.909	1.08	0.28	4.13
IL4R	4	rs10024216	A	0.33	0.427	0.86	0.59	1.25	A	0.45	0.561	0.75	0.28	1.98	A	0.47	0.983	1.01	0.46	2.20
IL4R	4	rs10004195	A	0.18	**0.02 A** 0.707 B **0.022 T**	0.58	0.37	0.92	A	0.39	0.793	0.87	0.31	2.45	A	0.50	0.423	1.39	0.62	3.13
SLC39A8	4	rs10014145	G	0.30	0.614	0.91	0.61	1.34	G	0.30	0.107	2.45	0.81	7.45	G	0.06	0.261	0.43	0.09	1.95
IL4	5	rs2243250	T	0.26	**0.010 A** 0.339 B **0.007 T**	1.72	1.14	2.59	C	0.50	0.729	1.19	0.45	3.12	C	0.19	0.432	0.68	0.26	1.80

			Caucasians (n=526)						Admixed (n=58)						East Asian (n=79)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
IL4	5	rs2070874	T	0.26	**0.009 A** 0.304 B **0.007 T**	1.73	1.14	2.61	C	0.50	0.719	1.19	0.47	3.00	C	0.19	0.432	0.68	0.26	1.80
IL4	5	rs2243268	C	0.26	**0.008 A** 0.292 B **0.006 T**	1.73	1.15	2.62	A	0.50	0.713	1.19	0.47	3.02	A	0.19	0.432	0.68	0.26	1.80
IL4	5	rs2243290	A	0.26	**0.011 A** 0.377 B **0.008 T**	1.70	1.13	2.57	C	0.45	0.980	0.99	0.39	2.51	C	0.19	0.432	0.68	0.26	1.80
IL4	5	rs12186803	A	0.25	**0.006 A** 0.196 B **0.004 T**	1.79	1.18	2.72	G	0.36	0.214	0.55	0.21	1.43	G	0.25	0.926	0.96	0.39	2.35
ADRB2	5	rs877741	C	0.19	0.538	0.87	0.55	1.37	C	0.45	0.565	0.76	0.30	1.94	T	0.13	0.094 A 0.135 T	0.39	0.13	1.21
HLA	6	rs7192	T	0.37	0.630	1.09	0.76	1.58	T	0.40	0.817	1.12	0.42	3.02	T	0.38	0.173	1.76	0.78	4.00
TYRP1	9	rs1952692	C	0.38	0.467	1.15	0.79	1.66	C	0.14	0.454	0.61	0.16	2.27	C	0.03	0.565	0.54	0.06	4.55
TLR4	9	rs7849955	A	0.11	0.375	0.78	0.45	1.35	A	0.05	0.629	0.59	0.07	5.08	A	0.00	NA	NA	NA	NA
TLR4	9	rs1930713	A	0.23	0.956	0.99	0.65	1.50	A	0.14	0.881	0.90	0.24	3.46	A	0.09	0.128	3.16	0.67	14.88
TLR4	9	rs2245960	T	0.18	0.938	1.02	0.64	1.61	T	0.09	0.634	0.68	0.14	3.30	T	0.09	0.065 A 0.059 T	4.24	0.81	22.10
GATA3	10	rs10905349	A	0.19	0.673	0.91	0.58	1.42	A	0.41	0.314	1.63	0.63	4.25	G	0.47	0.877	0.94	0.43	2.05
IFNG	12	rs2069705	G	0.29	0.429	0.85	0.58	1.26	A	0.41	0.688	0.82	0.32	2.12	A	0.22	0.221	0.57	0.23	1.42
PHF11	13	rs7329078	T	0.40	0.982	1.00	0.69	1.44	T	0.20	0.426	0.62	0.19	2.03	T	0.22	0.470	0.71	0.28	1.80
LIG4	13	rs1805388	A	0.19	0.108	1.47	0.92	2.34	A	0.30	0.363	1.65	0.56	4.86	A	0.30	0.165	1.90	0.76	4.73
IRS2	13	rs2289046	C	0.28	0.391	0.84	0.57	1.24	C	0.32	0.512	0.72	0.27	1.93	T	0.53	0.522	1.29	0.59	2.81
PTGER2	14	rs708491	G	0.22	0.206	0.75	0.49	1.17	G	0.10	0.309	2.44	0.42	14.37	G	0.00	NA	NA	NA	nan

			Caucasians (n=526)						Admixed (n=58)						East Asian (n=79)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
STAT5B	17	rs16967593	A	0.23	**0.031 A** 1 B **0.027 T**	0.64	0.42	0.96	A	0.32	0.697	1.22	0.45	3.33	A	0.53	0.197	1.67	0.76	3.64
TGFb1	19	rs2241712	C	0.36	0.858	1.03	0.71	1.50	C	0.50	0.231	1.77	0.69	4.50	T	0.41	0.736	0.87	0.39	1.93

A1 – minor allele of SNP; OR – odds ratio for allelic trait model, L95/U95 – lower and upper range of 95% confidence interval for allelic trait model; NA – not applicable as odds ratio could not be computed since there were no infants with the major allele within the population group

[i] For SNPs that showed some evidence of association (nominal $P < 0.1$), several other tests based on a genotypic trait model were carried out to test for an association. The p-values for these tests are given in the table. Bonferroni adjusted *P* values are given for SNPs with nominal *P* value <0.05.

[ii] A – allelic trait model, B – Bonferroni adjusted, G – genotypic trait model, T – trend/additive trait model, D – dominant trait model, R – recessive trait model. Default model of analyses is the allelic trait model.

[iii] ORs and CIs shown are associated with the default model of analyses - allelic trait model.

7.4.4 CTLA4 variants are associated with reduced risk of egg allergy among sensitised infants

The third set of analysis was carried out between the food sensitised tolerant infants (asymptomatic) and the food sensitised allergic infants (symptomatic). The objective of this analysis was to identify any variants that may increase the risk of food sensitised infants to develop food allergy.

Three SNPs in *CTLA4* (rs231735, rs231804, rs11571291) and rs10014145 in *SLC39A8* were all associated with a reduced risk of egg allergy in the East Asian population (Table 7.8). The associations for the three SNPs of *CTLA4* were attenuated after Bonferroni correction (P_{adjusted}=0.079 for rs231735 and P_{adjusted}=0.081 for rs231804 and rs11571291). Under the additive trait model, the three *CTLA4* SNPs were associated with egg allergy ($P_{unadjusted}$ <0.05).

While rs7192 (*HLA*) was associated with an increased risk of peanut allergy in Caucasian infants, there was no evidence that the risk allele increases the risk of peanut sensitised individuals to develop peanut allergy ($P_{unadjusted}$ =0.034; OR=1.68, 95% CI=1.04-2.71; P_{adjusted}>0.05) (Table 7.9).

Interestingly, when comparing Caucasian food sensitised tolerant infants to food allergy infants, rs2289046 of *IRS2*, was associated with an increased risk of food allergy ($P_{unadjusted}$<0.001; OR=2.57, CI=1.56-4.22). This association remained significant even after adjusting for multiple testing (P_{adjusted}=0.00475) (Table 7.10). This variant was not previously associated with food allergy (see Section 7.4.1, Table 7.2) and was found to only have weak evidence of association with any food sensitisation in Caucasian infants ($P_{unadjusted}$ =0.056; OR=0.72, 95% CI 0.51-1.01; P_{adjusted}>0.05).

Table 7.8 Association of investigated SNPs with egg allergy by parent's country of birth (egg sensitised tolerant vs egg allergics)

			Caucasians (n=259)						Admixed (n=30)						East Asian (n=54)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
ADAM17	2	rs10495562	T	0.50	0.344	1.25	0.79	1.99	C	0.22	0.197	NA	NA	NA	C	0.03	0.619	NA	NA	NA
ADAM17	2	rs1056204	C	0.31	0.445	0.83	0.51	1.35	C	0.13	0.348	NA	NA	NA	C	0.00	NA	NA	NA	NA
KCNS3	2	rs4368333	C	0.43	0.781	0.94	0.59	1.49	A	0.22	0.754	1.43	0.15	13.43	A	0.03	0.171	0.22	0.02	2.36
SOCS5	2	rs6737848	G	0.09	0.324	1.62	0.62	4.23	G	0.33	0.975	0.97	0.16	5.84	G	0.45	0.076 A 0.072 T	5.68	0.67	47.96
EDAR	2	rs6749207	C	0.16	0.214	1.59	0.76	3.33	T	0.33	0.092 A 0.110 T	NA	NA	NA	T	0.27	0.381	2.53	0.30	21.54
DPP10	2	rs4353658	A	0.41	0.153	0.71	0.45	1.14	A	0.37	0.858	1.18	0.20	7.01	A	0.15	0.848	1.24	0.14	10.78
DPP10	2	rs7579207	A	0.31	0.408	0.82	0.50	1.32	A	0.17	0.278	NA	NA	NA	A	0.04	0.564	NA	NA	NA
CTLA4	2	rs231735	G	0.46	0.131	1.44	0.90	2.32	G	0.39	0.791	1.27	0.21	7.57	G	0.17	**0.002 A** 0.081 B **0.008 T**	0.13	0.03	0.58
CTLA4	2	rs231804	C	0.42	0.274	1.31	0.81	2.13	C	0.35	0.928	1.09	0.18	6.48	C	0.16	**0.002 A** 0.079 B **0.007 T**	0.10	0.02	0.58
CTLA4	2	rs11571291	C	0.43	0.094	1.51	0.93	2.46	C	0.31	0.898	0.89	0.15	5.36	C	0.17	**0.002 A** 0.081 B **0.008 T**	0.13	0.03	0.58
IKZF2	2	rs12619285	G	0.23	0.829	0.94	0.55	1.62	G	0.44	0.297	0.40	0.07	2.36	A	0.39	0.440	1.90	0.36	9.90
CHL1	3	rs4684083	C	0.27	0.302	0.77	0.47	1.27	C	0.13	0.348	NA	NA	NA	C	0.08	0.672	0.62	0.07	5.71
CD200R1L	3	rs4682429	A	0.43	0.441	0.83	0.52	1.33	A	0.31	0.105 A 0.121 T	NA	NA	NA	A	0.08	0.406	NA	NA	NA
IL4R	4	rs10024216	A	0.36	0.589	1.15	0.70	1.88	A	0.44	0.627	1.56	0.26	9.32	A	0.52	0.575	0.65	0.15	2.90
IL4R	4	rs10004195	A	0.24	0.250	1.42	0.78	2.60	A	0.44	0.809	0.78	0.10	5.99	A	0.45	0.796	0.83	0.19	3.53
SLC39A8	4	rs10014145	G	0.32	0.910	0.97	0.59	1.61	G	0.22	0.754	1.43	0.15	13.43	G	0.12	**0.004 A** 0.119 B **0.003 T**	0.14	0.03	0.62

			Caucasians (n=259)						Admixed (n=30)						East Asian (n=54)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
IL4	5	rs2243250	T	0.20	0.482	0.82	0.47	1.43	C	0.42	0.673	1.47	0.25	8.74	C	0.18	0.694	1.54	0.18	13.28
IL4	5	rs2070874	T	0.20	0.451	0.81	0.46	1.41	C	0.43	0.663	1.48	0.25	8.81	C	0.18	0.694	1.54	0.18	13.28
IL4	5	rs2243268	C	0.19	0.420	0.80	0.46	1.39	A	0.43	0.663	1.48	0.25	8.81	A	0.18	0.694	1.54	0.18	13.28
IL4	5	rs2243290	A	0.20	0.389	0.78	0.45	1.37	C	0.39	0.284	3.18	0.35	29.17	C	0.19	0.649	1.64	0.19	14.15
IL4	5	rs12186803	A	0.18	0.411	0.79	0.45	1.39	G	0.37	0.321	2.94	0.32	27.00	G	0.20	0.606	1.75	0.20	15.05
ADRB2	5	rs877741	C	0.20	0.789	0.92	0.52	1.64	C	0.43	0.058 A 0.065 T	0.15	0.02	1.36	T	0.22	0.868	0.87	0.16	4.61
HLA	6	rs7192	T	0.36	0.699	0.91	0.56	1.47	T	0.44	0.602	1.60	0.27	9.49	T	0.25	1.000	1.00	0.19	5.28
TYRP1	9	rs1952692	C	0.32	0.101 A 0.127 G 0.093 T **0.044 D** 0.739 R	0.67	0.42	1.08	C	0.17	1.000	1.00	0.10	9.61	C	0.06	0.485	0.46	0.05	4.34
TLR4	9	rs7849955	A	0.15	0.770	1.10	0.57	2.15	A	0.07	0.490	NA	NA	NA	A	0.00	NA	NA	NA	NA
TLR4	9	rs1930713	A	0.22	0.979	1.01	0.58	1.76	A	0.13	0.348	NA	NA	NA	A	0.05	0.517	NA	NA	NA
TLR4	9	rs2245960	T	0.17	0.973	0.99	0.54	1.82	T	0.13	0.348	NA	NA	NA	T	0.05	0.517	NA	NA	NA
GATA3	10	rs10905349	A	0.22	0.792	1.08	0.61	1.90	A	0.35	0.361	2.71	0.30	24.95	G	0.48	0.567	1.54	0.35	6.79
IFNG	12	rs2069705	G	0.33	0.394	0.81	0.49	1.32	A	0.37	0.536	0.59	0.11	3.20	A	0.33	0.224	3.50	0.41	29.68
PHF11	13	rs7329078	T	0.40	0.547	1.16	0.72	1.88	T	0.20	0.830	1.28	0.14	12.10	T	0.22	0.844	0.85	0.16	4.49
LIG4	13	rs1805388	A	0.15	0.630	1.18	0.60	2.36	A	0.32	0.101 A 0.122 T	NA	NA	NA	A	0.27	0.380	2.54	0.30	21.66
IRS2	13	rs2289046	C	0.34	0.082 A **8.22E-04 G** 0.071 T **0.002 D** 0.200 R	1.60	0.94	2.71	C	0.33	**0.017 A** 0.590 B **0.022 T**	0.10	0.01	0.92	T	0.48	0.209	2.77	0.53	14.39
PTGER2	14	rs708491	G	0.26	0.426	1.27	0.70	2.32	G	0.08	0.472	NA	NA	NA	G	0.01	0.774	NA	NA	NA

			Caucasians (n=259)						Admixed (n=30)						East Asian (n=54)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
STAT5B	17	rs16967593	A	0.27	1 B **0.039 A** **0.034 T**	1.89	1.03	3.48	A	0.28	0.052 A 0.019 T	0.19	0.03	1.16	A	0.40	**0.05 A** 1 B 0.063 T	NA	NA	NA
TGFb1	19	rs2241712	C	0.37	0.632	0.88	0.54	1.46	C	0.38	0.184	0.31	0.05	1.87	T	0.44	0.271	0.39	0.07	2.22

A1 – minor allele of SNP; OR – odds ratio for allelic trait model, L95/U95 – lower and upper range of 95% confidence interval for allelic trait model; NA – not applicable as odds ratio could not be computed since there were no infants with the major allele within the population group

[i] For SNPs that showed some evidence of association (nominal $P < 0.1$), several other tests based on a genotypic trait model were carried out to test for an association. The p-values for these tests are given in the table. Bonferroni adjusted *P* values are given for SNPs with nominal *P* value <0.05.

[ii]A – allelic trait model, B – Bonferroni adjusted, G – genotypic trait model, T – trend/additive trait model, D – dominant trait model, R – recessive trait model. Default model of analyses is the allelic trait model.

[iii] ORs and CIs shown are associated with the default model of analyses - allelic trait model.

Table 7.9 Association of investigated SNPs with peanut allergy by parent's country of birth (peanut sensitised tolerant vs peanut allergics)

			Caucasians (n=139)						Admixed (n=24)						East Asian (n=33)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
ADAM17	2	rs10495562	T	0.44	0.945	0.98	0.61	1.58	C	0.15	0.796	0.82	0.18	3.74	C	0.06	0.164	NA	NA	NA
ADAM17	2	rs1056204	C	0.34	0.945	0.98	0.60	1.61	C	0.12	0.099 A 0.089 T	NA	NA	NA	C	0.03	0.328	NA	NA	NA
KCNS3	2	rs4368333	C	0.41	0.990	1.00	0.62	1.62	A	0.15	0.312	0.48	0.12	2.01	A	0.12	0.436	2.00	0.34	11.76
SOCS5	2	rs6737848	G	0.13	0.333	1.44	0.69	3.03	G	0.31	0.955	1.04	0.29	3.69	G	0.44	0.205	0.53	0.19	1.43
EDAR	2	rs6749207	C	0.11	0.087 A 0.104 T	0.55	0.28	1.10	T	0.21	0.165	0.39	0.10	1.49	T	0.16	0.227	0.47	0.14	1.61
DPP10	2	rs4353658	A	0.41	0.227	1.35	0.83	2.20	A	0.31	0.464	0.64	0.20	2.11	A	0.18	0.908	0.93	0.27	3.25
DPP10	2	rs7579207	A	0.37	0.234	1.35	0.82	2.23	A	0.15	0.796	0.82	0.18	3.74	A	0.06	0.591	1.94	0.17	22.47
CTLA4	2	rs231735	G	0.53	0.463	1.19	0.74	1.92	G	0.46	0.493	1.50	0.47	4.79	G	0.21	0.244	2.33	0.55	9.99
CTLA4	2	rs231804	C	0.49	0.340	1.26	0.78	2.03	C	0.46	0.312	1.84	0.56	6.00	C	0.19	0.491	1.62	0.41	6.38
CTLA4	2	rs11571291	C	0.50	0.286	1.30	0.80	2.08	C	0.42	0.455	1.57	0.48	5.15	C	0.21	0.378	1.82	0.48	6.91
IKZF2	2	rs12619285	G	0.25	0.636	0.88	0.51	1.51	G	0.38	0.863	0.90	0.28	2.88	A	0.44	0.774	1.15	0.43	3.07
CHL1	3	rs4684083	C	0.20	0.082 A 0.079 T	0.61	0.35	1.07	C	0.12	0.827	0.83	0.15	4.58	C	0.06	0.641	0.64	0.10	4.14
CD200R1L	3	rs4682429	A	0.46	0.314	1.28	0.79	2.05	A	0.35	0.585	1.41	0.41	4.87	A	0.06	0.592	0.60	0.09	3.88
IL4R	4	rs10024216	A	0.30	0.592	0.87	0.52	1.45	A	0.23	0.126	0.36	0.10	1.36	A	0.53	0.617	1.28	0.48	3.43
IL4R	4	rs10004195	A	0.18	0.985	1.01	0.54	1.88	A	0.38	0.927	0.94	0.27	3.32	A	0.41	0.479	0.70	0.26	1.88
SLC39A8	4	rs10014145	G	0.30	0.938	0.98	0.58	1.65	G	0.35	0.741	1.24	0.35	4.32	G	0.18	0.156 A 0.127 T	3.21	0.60	17.27
IL4	5	rs2243250	T	0.19	0.211	0.70	0.39	1.23	C	0.38	0.434	0.63	0.19	2.03	C	0.15	0.660	0.75	0.20	2.74
IL4	5	rs2070874	T	0.19	0.159	0.66	0.37	1.18	C	0.38	0.422	0.63	0.20	1.97	C	0.15	0.660	0.75	0.20	2.74
IL4	5	rs2243268	C	0.19	0.159	0.66	0.37	1.18	A	0.38	0.422	0.63	0.20	1.97	A	0.15	0.660	0.75	0.20	2.74
IL4	5	rs2243290	A	0.19	0.179	0.68	0.38	1.20	C	0.31	0.295	0.53	0.16	1.74	C	0.18	0.908	0.93	0.27	3.25

			Caucasians (n=139)						Admixed (n=24)						East Asian (n=33)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
IL4	5	rs12186803	A	0.18	0.151	0.65	0.37	1.17	G	0.31	0.682	0.78	0.23	2.59	G	0.21	0.669	0.78	0.25	2.47
ADRB2	5	rs877741	C	0.24	0.289	1.37	0.76	2.47	C	0.29	0.253	0.49	0.15	1.67	T	0.29	0.093 A 0.108 T	2.92	0.81	10.50
HLA	6	rs7192	T	0.50	**0.034 A** 1 B 0.110 G **0.039 T** 0.117 D 0.062 R	1.68	1.04	2.71	T	0.50	0.500	1.50	0.46	4.88	T	0.29	0.486	0.69	0.25	1.94
TYRP1	9	rs1952692	C	0.31	0.206	0.72	0.44	1.20	C	0.15	0.864	1.15	0.23	5.81	C	0.00	0.299	0.00	0.00	nan
TLR4	9	rs7849955	A	0.19	0.076 A 0.068 T	1.84	0.93	3.61	A	0.08	0.654	1.75	0.15	20.71	A	0.03	0.314	NA	NA	NA
TLR4	9	rs1930713	A	0.25	0.738	1.10	0.63	1.91	A	0.12	0.827	0.83	0.15	4.58	A	0.06	0.592	0.60	0.09	3.88
TLR4	9	rs2245960	T	0.17	0.803	0.92	0.50	1.72	T	0.12	0.782	1.30	0.20	8.61	T	0.06	0.592	0.60	0.09	3.88
GATA3	10	rs10905349	A	0.26	0.140	1.53	0.87	2.71	A	0.46	0.715	1.24	0.39	3.90	G	0.41	0.641	0.79	0.30	2.10
IFNG	12	rs2069705	G	0.36	0.217	1.38	0.83	2.28	A	0.29	0.404	0.59	0.18	2.02	A	0.22	1.000	1.00	0.31	3.27
PHF11	13	rs7329078	T	0.37	0.577	0.87	0.53	1.42	T	0.23	0.802	1.20	0.29	4.99	T	0.22	1.000	1.00	0.31	3.27
LIG4	13	rs1805388	A	0.15	0.384	0.75	0.39	1.43	A	0.13	0.152	0.33	0.07	1.56	A	0.29	0.959	0.97	0.33	2.85
IRS2	13	rs2289046	C	0.37	0.150	1.45	0.87	2.40	C	0.50	0.203	2.14	0.66	6.98	T	0.47	0.622	0.78	0.30	2.06
PTGER2	14	rs708491	G	0.26	0.427	1.26	0.71	2.25	G	0.17	0.521	1.80	0.29	11.03	G	0.03	0.344	NA	NA	NA
STAT5B	17	rs16967593	A	0.23	0.966	1.01	0.58	1.77	A	0.31	0.938	0.95	0.28	3.24	A	0.26	**0.027 A** 0.937 B **0.017 T**	0.32	0.11	0.89
TGFb1	19	rs2241712	C	0.28	0.163	0.69	0.42	1.16	C	0.54	0.778	1.18	0.37	3.77	T	0.47	0.614	1.29	0.48	3.47

A1 – minor allele of SNP; OR – odds ratio for allelic trait model, L95/U95 – lower and upper range of 95% confidence interval for allelic trait model; NA – not applicable as odds ratio could not be computed since there were no infants with the major allele within the population group

[i] For SNPs that showed some evidence of association (nominal $P < 0.1$), several other tests based on a genotypic trait model were carried out to test for an association. The p-values for these tests are given in the table. Bonferroni adjusted *P* values are given for SNPs with nominal *P* value <0.05.

[ii]A – allelic trait model, B – Bonferroni adjusted, G – genotypic trait model, T – trend/additive trait model, D – dominant trait model, R – recessive trait model. Default model of analyses is the allelic trait model.

[iii] ORs and CIs shown are associated with the default model of analyses - allelic trait model.

Table 7.10 Association of investigated SNPs with 'any food allergy' by parent's country of birth (food sensitised tolerant vs food allergics)

			Caucasians (n=306)						Admixed (n=36)						East Asian (n=60)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
ADAM17	2	rs10495562	T	0.49	0.410	1.18	0.80	1.75	C	0.20	0.338	2.75	0.32	23.43	C	0.04	0.498	NA	NA	NA
ADAM17	2	rs1056204	C	0.31	0.646	0.91	0.60	1.38	C	0.12	0.213	NA	NA	NA	C	0.01	0.738	NA	NA	NA
KCNS3	2	rs4368333	C	0.43	0.762	1.06	0.71	1.58	A	0.20	0.697	0.75	0.18	3.20	A	0.06	0.402	NA	NA	NA
SOCS5	2	rs6737848	G	0.09	0.635	1.19	0.58	2.43	G	0.31	0.876	0.90	0.24	3.38	G	0.44	0.353	0.57	0.17	1.90
EDAR	2	rs6749207	C	0.15	0.602	1.17	0.65	2.09	T	0.31	0.498	0.64	0.18	2.32	T	0.27	0.862	1.13	0.29	4.47
DPP10	2	rs4353658	A	0.41	0.881	0.97	0.65	1.45	A	0.37	0.826	1.16	0.31	4.29	A	0.15	0.541	1.91	0.23	15.86
DPP10	2	rs7579207	A	0.31	0.571	0.89	0.59	1.34	A	0.15	0.884	0.88	0.17	4.71	A	0.05	0.446	NA	NA	NA
CTLA4	2	rs231735	G	0.46	0.374	1.20	0.81	1.78	G	0.37	0.744	0.81	0.23	2.86	G	0.20	0.083 A 0.130 T	0.35	0.10	1.20
CTLA4	2	rs231804	C	0.42	0.521	1.14	0.76	1.72	C	0.33	0.580	0.70	0.20	2.49	C	0.18	0.058 A 0.092 T	0.31	0.09	1.09
CTLA4	2	rs11571291	C	0.44	0.253	1.27	0.84	1.90	C	0.29	0.401	0.58	0.16	2.09	C	0.20	0.083 A 0.13 T	0.35	0.10	1.20
IKZF2	2	rs12619285	G	0.23	0.232	0.76	0.49	1.19	G	0.45	0.841	1.14	0.32	4.01	A	0.39	0.448	0.63	0.19	2.09
CHL1	3	rs4684083	C	0.28	0.272	0.79	0.52	1.20	C	0.13	0.761	0.77	0.14	4.17	C	0.08	0.294	NA	NA	NA
CD200R1L	3	rs4682429	A	0.43	0.818	0.95	0.64	1.42	A	0.30	0.728	1.29	0.31	5.31	A	0.07	0.908	0.88	0.10	7.71
IL4R	4	rs10024216	A	0.36	0.382	1.21	0.79	1.84	A	0.41	0.586	0.69	0.18	2.66	A	0.51	0.631	0.74	0.22	2.50
IL4R	4	rs10004195	A	0.23	0.624	1.13	0.70	1.83	A	0.43	0.310	0.49	0.13	1.96	A	0.45	0.722	0.80	0.24	2.68
SLC39A8	4	rs10014145	G	0.32	0.589	1.13	0.73	1.75	G	0.27	0.655	1.46	0.28	7.59	G	0.14	0.794	0.81	0.16	4.05
IL4	5	rs2243250	T	0.20	0.450	0.83	0.52	1.34	C	0.41	0.985	0.99	0.28	3.49	C	0.19	0.378	2.50	0.30	20.50
IL4	5	rs2070874	T	0.20	0.418	0.82	0.51	1.32	C	0.42	1.000	1.00	0.28	3.52	C	0.19	0.378	2.50	0.30	20.50
IL4	5	rs2243268	C	0.20	0.388	0.81	0.51	1.30	A	0.42	1.000	1.00	0.28	3.52	A	0.19	0.378	2.50	0.30	20.50
IL4	5	rs2243290	A	0.20	0.431	0.83	0.52	1.33	C	0.37	0.744	0.81	0.23	2.86	C	0.20	0.315	2.81	0.34	22.98
IL4	5	rs12186803	A	0.19	0.348	0.80	0.49	1.28	G	0.35	0.661	0.75	0.21	2.67	G	0.21	0.708	1.35	0.28	6.61

			Caucasians (n=306)						Admixed (n=36)						East Asian (n=60)					
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
ADRB2	5	rs877741	C	0.21	0.421	1.23	0.74	2.06	C	0.43	0.662	0.76	0.22	2.63	T	0.25	0.507	0.65	0.18	2.34
HLA	6	rs7192	T	0.37	0.672	0.92	0.61	1.37	T	0.45	0.375	1.91	0.45	8.10	T	0.27	0.280	0.51	0.15	1.75
TYRP1	9	rs1952692	C	0.32	0.084 A 0.21 G 0.084 T 0.17 D 0.136 R	0.70	0.46	1.05	C	0.17	NA	NA	NA	NA	C	0.06	0.710	0.66	0.07	6.00
TLR4	9	rs7849955	A	0.16	0.423	1.27	0.71	2.26	A	0.07	0.836	0.79	0.08	7.72	A	0.01	0.735	NA	NA	NA
TLR4	9	rs1930713	A	0.22	0.358	1.26	0.77	2.09	A	0.12	0.737	1.45	0.16	13.03	A	0.05	0.577	0.53	0.06	4.99
TLR4	9	rs2245960	T	0.17	0.642	1.14	0.66	1.96	T	0.12	0.213	NA	NA	NA	T	0.05	0.577	0.53	0.06	4.99
GATA3	10	rs10905349	A	0.23	0.267	1.33	0.80	2.18	A	0.40	0.665	1.33	0.36	4.93	G	0.48	0.329	1.86	0.53	6.54
IFNG	12	rs2069705	G	0.32	0.347	0.82	0.54	1.24	A	0.34	**0.038 A** 1 B **0.043 T**	0.26	0.07	0.98	A	0.33	0.082 A 0.128 T	5.34	0.66	43.10
PHF11	13	rs7329078	T	0.40	0.460	0.86	0.57	1.29	T	0.22	0.905	1.11	0.21	5.86	T	0.23	0.409	0.59	0.16	2.11
LIG4	13	rs1805388	A	0.15	0.460	1.25	0.69	2.27	A	0.29	0.396	2.00	0.39	10.16	A	0.25	0.186	3.76	0.46	30.58
IRS2	13	rs2289046	C	0.35	**1.4×10^{-4} A** **0.005 B** **8.9×10^{-5} T**	2.57	1.56	4.22	C	0.37	0.162	0.41	0.12	1.46	T	0.47	0.714	1.25	0.37	4.19
PTGER2	14	rs708491	G	0.26	0.920	1.03	0.64	1.64	G	0.07	0.291	0.38	0.06	2.39	G	0.02	0.631	NA	NA	NA
STAT5B	17	rs16967593	A	0.26	0.299	1.28	0.80	2.06	A	0.28	0.142	0.40	0.11	1.40	A	0.39	0.945	0.95	0.25	3.58
TGFb1	19	rs2241712	C	0.36	0.854	1.04	0.68	1.59	C	0.43	0.137	0.38	0.10	1.40	T	0.44	0.878	1.10	0.33	3.70

A1 – minor allele of SNP; OR – odds ratio for allelic trait model, L95/U95 – lower and upper range of 95% confidence interval for allelic trait model; NA – not applicable as odds ratio could not be computed since there were no infants with the major allele within the population group

[i] For SNPs that showed some evidence of association (nominal $P < 0.1$), several other tests based on a genotypic trait model were carried out to test for an association. The p-values for these tests are given in the table. Bonferroni adjusted *P* values are given for SNPs with nominal *P* value <0.05.

[ii]A – allelic trait model, B – Bonferroni adjusted, G – genotypic trait model, T – trend/additive trait model, D – dominant trait model, R – recessive trait model. Default model of analyses is the allelic trait model.

[iii] ORs and CIs shown are associated with the default model of analyses - allelic trait model.

7.4.5 *KIF3A/IL4* variants may be associated with food allergy in infants with East Asian parents

The earlier analyses carried out in this chapter in East Asian population were restricted to only infants with both parents born in East Asia to obtain a neat group of East Asians. However, due to the small sample size of the East Asian population in HealthNuts, another set of analyses were carried out in a combined East Asian population consisting of infants with either one or both parents of East Asian descent. This was done primarily to increase the power of these analyses to detect an association.

None of the investigated SNPs were uniquely and strongly associated with any food allergy or any food sensitisation in the combined East Asian population under the allelic trait model, after correction for multiple testing (Table 7.11). Noteworthy, however, is the G allele of rs12186803 *(KIF3A)*, which showed some evidence of association with any food allergy after multiple testing adjustment ($P_{unadjusted}$=0.001; OR=0.46, 95% CI=0.29-0.75; $P_{adjusted}$=0.052).

When analysed under different trait models, *IL4* variants, (rs2243250, rs2070874, rs2243268, rs2243290, rs12186803) were all associated with any food allergy in the additive, dominant and genotypic models (Table 7.11). In particular, rs12186803 was also associated with any food sensitisation under the additive model ($P_{unadjusted}$=0.018) (Table 7.11). Interestingly, none of the *IL4* variants were associated with any food allergy in the Caucasian infants (Table 7.2). The SNPs were however associated with food sensitisation as discussed in Section 7.4.3 (Table 7.5).

Table 7.11 Association of investigated SNPs with 'any food allergy' (non-atopic controls vs food allergic) and 'any food sensitisation' (non-atopic controls vs food sensitised tolerant) in infants with one or both parents born in East Asia

				Any food allergy (n=86)					Any food sensitisation (n=41)				
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
ADAM17	2	rs10495562	C	0.09524	0.459	0.76	0.37	1.57	0.06757	0.217	0.52	0.19	1.48
ADAM17	2	rs1056204	C	0.04762	0.923	0.95	0.34	2.69	0.01351	0.181	0.26	0.03	2.16
KCNS3	2	rs4368333	A	0.1071	**0.044 A** 1 B **0.043 T**	0.52	0.27	0.99	0.1216	0.212	0.60	0.26	1.35
SOCS5	2	rs6737848	G	0.3951	0.216	1.35	0.84	2.17	0.4286	0.146	1.55	0.86	2.80
EDAR	2	rs6749207	T	0.2875	0.072	0.64	0.40	1.04	0.2639	0.077	0.57	0.31	1.07
DPP10	2	rs4353658	A	0.2262	0.790	0.93	0.55	1.58	0.2568	0.776	1.10	0.57	2.11
DPP10	2	rs7579207	A	0.08333	0.940	0.97	0.43	2.17	0.06757	0.641	0.77	0.26	2.28
CTLA4	2	rs231735	G	0.259	0.311	0.77	0.47	1.27	0.2639	0.472	0.79	0.42	1.50
CTLA4	2	rs231804	C	0.2378	0.376	0.79	0.47	1.33	0.25	0.614	0.85	0.44	1.62
CTLA4	2	rs11571291	C	0.2317	0.349	0.78	0.47	1.31	0.2568	0.733	0.89	0.47	1.70
IKZF2	2	rs12619285	A	0.4451	0.565	0.88	0.56	1.38	0.4595	0.794	0.93	0.53	1.63
CHL1	3	rs4684083	C	0.1024	0.341	1.48	0.66	3.35	0.1081	0.358	1.58	0.59	4.18
CD200R1L	3	rs4682429	A	0.1548	0.954	0.98	0.53	1.82	0.1216	0.483	0.74	0.32	1.71
IL4R	4	rs10024216	A	0.474	0.772	1.07	0.68	1.69	0.4583	0.987	1.01	0.57	1.78
IL4R	4	rs10004195	A	0.4384	0.279	1.30	0.81	2.10	0.4559	0.267	1.40	0.77	2.52
SLC39A8	4	rs10014145	G	0.1845	0.327	1.36	0.74	2.51	0.1806	0.473	1.32	0.62	2.84
IL4	5	rs2243250	C	0.2651	**0.017 A** 0.605 B **0.023 G** **0.023 T** **0.006 D** 0.520 R	0.56	0.34	0.90	0.2917	0.146	0.64	0.35	1.17

				Any food allergy (n=86)					Any food sensitisation (n=41)				
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
IL4	5	rs2070874	C	0.2679	**0.020 A** 0.688 B **0.028 G** **0.025 T** **0.008 D** 0.502 R	0.57	0.35	0.91	0.2973	0.166	0.65	0.36	1.20
IL4	5	rs2243268	A	0.2679	**0.022 A** 0.757 B **0.035 G** **0.028 T** **0.01 D** 0.481 R	0.57	0.35	0.92	0.2973	0.174	0.66	0.36	1.21
IL4	5	rs2243290	C	0.2619	**0.02 A** 0.707 B 0.061 G **0.023 T** **0.02 D** 0.241 R	0.57	0.35	0.92	0.2703	0.091	0.59	0.32	1.09
IL4	5	rs12186803	G	0.2619	**0.001 A** 0.052 B **0.004 G** **0.002 T** **9.55x10^{-4} D** 0.151 R	0.46	0.29	0.75	0.2703	**0.019 A** 0.647 B **0.018 T**	0.48	0.26	0.89
ADRB2	5	rs877741	T	0.3598	0.453	1.20	0.74	1.96	0.3243	0.928	1.03	0.56	1.89
HLA	6	rs7192	T	0.3333	0.965	0.99	0.62	1.59	0.375	0.570	1.19	0.66	2.15
TYRP1	9	rs1952692	C	0.09639	0.348	0.71	0.35	1.45	0.08108	0.280	0.59	0.22	1.55
TLR4	9	rs7849955	A	0.03012	0.372	0.59	0.18	1.90	0.01351	0.181	0.26	0.03	2.16
TLR4	9	rs1930713	A	0.07143	0.270	0.64	0.29	1.42	0.08108	0.542	0.74	0.27	1.98
TLR4	9	rs2245960	T	0.07143	0.641	0.82	0.36	1.89	0.06757	0.641	0.77	0.26	2.28
GATA3	10	rs10905349	A	0.4762	0.478	1.18	0.75	1.85	0.473	0.602	1.16	0.66	2.05
IFNG	12	rs2069705	A	0.3333	0.792	0.94	0.58	1.51	0.2838	0.343	0.74	0.40	1.37

				Any food allergy (n=86)					Any food sensitisation (n=41)				
Gene	**CHR**	**SNP**	**A1**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**	**MAF Cases**	**P[i, ii]**	**OR[iii]**	**L95**	**U95**
PHF11	13	rs7329078	T	0.2229	0.162	0.69	0.41	1.16	0.1944	0.122	0.58	0.29	1.16
LIG4	13	rs1805388	A	0.2658	**0.05 A** 1 B 0.077 T	1.78	1.00	3.17	0.2429	0.210	1.58	0.77	3.21
IRS2	13	rs2289046	C	0.4702	0.819	1.05	0.67	1.65	0.527	0.331	1.32	0.75	2.33
PTGER2	14	rs708491	G	0.03704	0.990	1.01	0.30	3.38	0.02857	0.759	0.77	0.15	4.08
STAT5B	17	rs16967593	A	0.3512	0.878	1.04	0.65	1.66	0.4167	0.291	1.37	0.76	2.46
TGFb1	19	rs2241712	C	0.5127	0.276	1.29	0.82	2.04	0.5417	0.203	1.45	0.82	2.57

A1 – minor allele of SNP; OR – odds ratio for allelic trait model, L95/U95 – lower and upper range of 95% confidence interval for allelic trait model; NA – not applicable as odds ratio could not be computed since there were no infants with the major allele within the population group

[i] For SNPs that showed some evidence of association (nominal $P < 0.1$), several other tests based on a genotypic trait model were carried out to test for an association. The p-values for these tests are given in the table. Bonferroni adjusted *P* values are given for SNPs with nominal *P* value <0.05.

[ii] A – allelic trait model, B – Bonferroni adjusted, G – genotypic trait model, T – trend/additive trait model, D – dominant trait model, R – recessive trait model. Default model of analyses is the allelic trait model.

[iii] ORs and CIs shown are associated with the default model of analyses - allelic trait model.

7.5 Discussion

This chapter has investigated variants associated with food sensitisation, food allergy and its subtypes (peanut and egg allergy), stratified by parent's country of birth. *STAT5B* and *HLA* variants were associated with food allergy in the Caucasian infants, with some evidence for an association between food allergy and *KIF3A/IL4* and *CTLA4* variants in the East Asian infants. Despite these SNPs being associated with helminth diversity based on current literature, a majority of the associated SNPs were not more common in the East Asian population than the Caucasian population. This is contrary to our initial hypothesis of pro-inflammatory SNPs being more common in those with East Asian ancestry, therefore predisposing them to food allergy.

We were able to reproduce findings from the first GWAS in food allergy carried out by Hong et al (118) showing the association between peanut allergy and rs7192 (*HLA*), a non-synonymous SNP in the Caucasian infants. The association remained when tested under the different trait models. Under the allelic model, the T allele of rs7192 was associated with an increased risk of peanut allergy. There was no evidence of an association for this variant in the East Asian population in this thesis as well as previous GWAS carried out by Hong et al (118). The systematic review presented in Chapter 5, also reported similar associations for rs7192. The associated T allele in the HealthNuts population was also more common in the Caucasian population (MAF=0.5) than in the East Asian population (MAF=0.29). It is thought that rs7192 affects expression levels of HLA-DRB1 and HLA-DQB1 genes by influencing DNA methylation (252). The variant rs7192 has been discussed at length in the systematic review presented in Chapter 5.

Apart from rs7192 in *HLA*, rs16967593, an intron variant of *STAT5B* was also associated with food allergy (for all trait models) and food sensitisation (for all trait models except recessive) in the Caucasian population. Infants with the minor allele had reduced odds of any food allergy and any food sensitisation compared to the non-atopic infants. Variant rs16967593 was previously investigated for an association with atopic bronchial asthma in 428 ethnically Russian people with or without *Opisthorchis felineus* liver fluke invasion (317). However, in the study of Russian population, there was no evidence of association between the SNP and bronchial asthma.

While it is not yet established in the current literature whether rs16967593 is relevant to food allergy, STAT5B gene has been implicated in the pathogenesis of atopic dermatitis (322). In the genetic association analyses component of the study by Ando et al (2014), *STAT5B* SNP rs9900213 was significantly associated with a reduction in the risk of atopic dermatitis among European American subjects. Additionally, STAT5B loss of function variants have been shown to alter tolerance failure, while gain of function variants are known to alter cytokine signalling (323).

The protein encoded by *STAT5B* is a member of the STAT family of transcription factors and plays a key role in T cell differentiation (324-326). Studies then showed the role of this protein in mediating signal transduction (325, 327) and activating pathways in mast cells of patients with atopic dermatitis. It is thought that the silencing of the expression of *STAT5A* and *STAT5B*, resulted in mast cell apoptosis, thereby giving rise to the development of atopic dermatitis (322). Given the potential shared genetic makeup between atopic dermatitis and food allergy, it is biologically plausible that the association seen between rs16967593 and food allergy may be acting via mechanisms of development of atopic dermatitis. As this thesis did not address atopic dermatitis as an outcome, we are unable to verify if this particular *STAT5B* variant is also associated with atopic dermatitis in our population. This presents a potential future research that can be done to assess for any common associations between atopic dermatitis and food allergy in our population.

We also found weak evidence of an association between *CTLA4* variants and egg allergy/egg sensitisation among East Asian infants. Compared to the East Asian, non-atopic controls, egg sensitised tolerant infants carrying the G allele of rs231735 and C allele of rs231804 and rs11571291 were associated with a reduced risk of disease under the allelic model. Variant rs231804 was also associated with a reduced risk of egg allergy. When the egg sensitised tolerant (asymptomatic) infants were compared to the egg allergic infants (symptomatic), all three variants were significantly associated with a reduction in the risk of food allergy. Overall evidence following multiple testing adjustment was moderate suggesting larger sample sizes may be needed to robustly determine an association. Interestingly, variants rs231735, rs231804 and rs11571291 were also associated with peanut allergy in Caucasian infants. The associated alleles were all more common in Caucasians than East Asians infants.

Our findings for the association between *CTLA4* and food allergy or sensitisation are supported by existing studies. *CTLA4* can be found on the surface of activated Tregs and encodes for a glycoprotein receptor of the immunoglobulin family (141, 328-331). Its presence in Tregs indicates its crucial role in oral tolerance induction and suppression of sensitisation (332). Due to its significant influence in suppression of T cells activity (330, 333, 334), CTLA4 signaling negatively regulates immune activation and T cell proliferation (329, 335-337). Further, *CTLA4* represents a potential key therapeutic target for the prevention of induction and/or progression of allergic sensitisation, with evidence that *CTLA4* variants affects the Th1/Th2 balance (338). A study carried out in a peanut allergy murine model showed that *CTLA4* signaling was not a determining factor for development of food sensitisation but instead it mediates the intensity of responses to sensitisation (334). The transcription start site of *CTLA4* has also been shown to strongly correlate with helminth diversity (141). Additionally, *CTLA4* has been known to be involved in Th2 effector cell responses in the presence of helminth infections(339).

A major strength of this study is the objective measure of phenotype defined by an OFC and the capacity to investigate associations between the asymptomatic and symptomatic infants. This allows the ability to untangle whether any associations observed may be acting via different mechanisms in the symptomatic infants compared to asymptomatic infants. However, limitations within this study warrants careful consideration of results and their interpretation. Undoubtedly, the small East Asian sample size present a major weakness. Further replication of the results in a larger and independent population is necessary to confirm these findings. To partially address the limitation of the small sample size, a sub-analysis on a combined group of East Asian infants was carried out. There appears to be promising but weak evidence of association between *IL4/KIF3A* and food allergy. Future studies should therefore aim to address this in a larger cohort.

7.6 Conclusion

With the aim of identifying novel SNPs associated with food allergy in the East Asian population, the results from this chapter showed suggestive evidence of unique food allergy SNPs that are specific to East Asian population. There was a trend towards an association between variants in *CTLA4* and egg allergy/sensitisation in the East Asian population. The lack of a strong association may be a product of the small sample size of this

population in this study. We were able to confirm previous findings of rs7192 (*HLA*) association with peanut allergy only in the Caucasian population. Additionally, this study found a novel association between *STAT5B* and food allergy in the Caucasian population. Apart from these SNPs, there do not appear to be a strong association between food allergy and helminth-related SNPs. Based on the available sample, there are no striking differences in association patterns of genetic variants between Caucasians and East Asians. Further functional studies as well as studies of larger sample size are necessary to verify the findings obtained herein.

Taken together, these results argue for more specific attention on diverse (non-Caucasian) populations to fully understand the genetic mechanisms of food allergy. In particular, the Asian population is generally under represented in GWAS. Although our investigations so far has been on a small scale, this may represent one of the largest in the world with gold standard diagnosis of food allergy in ethnically diverse population, genotyped for food allergy variants.

Chapter 8 Overall discussion

The overall aim of this thesis is to expand existing knowledge on the prevalence and risk factors for food allergy in Asian children living in Melbourne. The research questions addressed in this thesis were described in section 1.9. In this chapter, a summary of the key findings is presented in section 8.1. A discussion of key findings and implications of the study are provided in sections 8.2 and 8.3. This is followed by a discussion on the strengths and limitations of the study in section 8.4. Finally, the future investigations and conclusions derived from this study are provided in sections 8.5 and 8.6, respectively.

8.1 Summary of key findings

8.1.1 Chapter 3

While we know from current literature that there are differences in food allergy prevalence between East Asian and Caucasian infants living in Australia, it is unclear if these differences persist into later childhood and whether other allergic diseases such as asthma, eczema and allergic rhinitis are also affected. This thesis explored these questions using data from the HealthNuts study and found:

- Children of East Asian-born parents had more allergic rhinitis and aeroallergen sensitisation at age 6 compared to children with Caucasian-born parents.
- Asthma was similar in both groups of children (those with East Asian-born parents and Caucasian-born parents).
- Children with IgE-mediated food allergy and eczema in infancy were 3 times more likely to have asthma and 2 times more likely to have allergic rhinitis at age 6, irrespective of ancestry.

8.1.2 Chapter 4

The results from the earlier Chapter 3 highlighted the higher prevalence of eczema, allergic rhinitis and food allergy in East Asians compared to Caucasian children. Given that the differences between the two populations living in Melbourne had been established in

the previous chapter, this chapter explored whether there were differences in the prevalence and risk factors for food allergy among East Asians living in different geographical locations – Singapore and Melbourne. In this chapter, it was reported that:

- Prevalence of food allergy among East Asian children living in Melbourne is 15.3% compared to 2.4% in Singapore.
- Higher proportion of GUSTO children with delayed introduction of solids, egg and peanut and shorter exclusive breastfeeding duration.
- Higher proportion of HealthNuts children with a family history of atopy.
- More HealthNuts mothers reported intake of allergenic foods during pregnancy compared to GUSTO mothers.
- Childcare attendance in the first 12 months of life was more common in HealthNuts compared with GUSTO.
- Eczema rates were lower in GUSTO than in HealthNuts. Age of eczema diagnosis was associated with an increased risk of food allergy in both HealthNuts and GUSTO, but the association did not differ by study.
- Cat ownership was associated with an increased risk of food allergy in the HealthNuts study.
- There is a trend towards delayed egg introduction associated with an increased risk of food allergy. Despite delayed introduction of food allergens into the infant diet compared to the HealthNuts infants, GUSTO infants had less food allergy.

8.1.3 Chapters 5 and 6

Apart from environmental risk factors, genetic risk factors are also known to contribute to the pathogenesis of food allergy. The effect of genetic factors may also differ depending on the environment. The fact that East Asian children in Melbourne have a higher rate of allergy compared to Caucasian children in Melbourne (despite a similar environment) indicate the presence of gene - environment interaction. Therefore, these two chapters sought to identify genetic factors that might explain the high rates of food allergy in East Asian children in the Australian environment.

Candidate gene studies and GWAS to date tended to focus on asthma or allergy in general and not on food allergy specifically. The scope of literature that exist on genetics of food allergy alone had not previously been systematically reviewed. With this in mind, this thesis systematically reviewed genetic studies on food allergy and curated a list of candidate genes and SNPs of interest to genotype in the Asian samples obtained from the HealthNuts study. This thesis found:

- A systematic literature search revealed that current studies were of varied quality.
- Few studies investigated the same set of SNPs for direct comparison to be made or meta-analyses to be carried out.
- Few of these studies were carried out in Asian population, a majority were carried out in Caucasian population.
- Several genes of interest known to be involved in immune regulation, cell function and epidermal barrier function were implicated in food allergy.
- Some of the highly reproducible genes with identified for an association with food allergy include *HLA, FLG* and *IL13*.
- Curated list of SNPs to genotype in HealthNuts samples: included 37 SNPs corresponding to 28 genes.

8.1.4 Chapter 7

Based on the list of SNPs shortlisted in the previous chapter (Chapter 6), genotyping and statistical analyses were carried out to explore the association between the candidate genes and food allergy. I was interested in exploring these effects in the Asian population particularly since existing genetic studies tend to limit their investigations to the Caucasian population. From the analyses carried out in the HealthNuts sample, this chapter reported that the:

- Minor allele of rs16967593 (*STAT5B)* was associated with a reduced risk of food allergy and food sensitisation in Caucasian infants.
- *HLA* rs7192 minor allele was associated with increased risk of peanut allergy in the Caucasian population but not East Asian population.

- Among sensitised children with two East Asian born parents, those with the minor allele for rs231735, rs231804 or rs11571291 (all *CTLA4*) have a reduced risk of egg allergy.

8.2 Implications of results – environmental aspect

This thesis has shown that children with East Asian born parents not only have more food allergy at 12 months but also allergic rhinitis, aeroallergen sensitisation and eczema at age 6 years, compared to children with Caucasian-born parents. When comparing food allergy prevalence of children with East Asian parents who are living in different geographical locations, the prevalence was higher in Australia than in Singapore. However, this thesis was unable to identify a key factor driving the disparity in food allergy risk between the Asian children in Australia and those in Singapore. It is likely that there may be other important contributing risk factors not captured in the studies included in this thesis and it is perhaps necessary to go beyond epidemiological comparison to uncover the underlying reasons.

Although the findings did not identify a key factor associated with the "Western environment", the prevalence of eczema was found to be higher in Asian children living in Australia than Asian children in Singapore. Notably, among children in Australia, those with Asian ancestry were more likely to be atopic than those with Caucasian ancestry. It is also in the Asian children that we see a high prevalence of food allergy. This points to the potentially seminal influence eczema may have on the development of food allergy.

Eczema is well-known to be co-associated with food allergy and together may lead to the progression of other allergic diseases such as allergic rhinitis and asthma (36, 45). The co-existence of these allergic diseases have been attributed to their shared genetic (40-44) and/or environmental factors including familial factors (168, 340, 341). However, it is unclear and is still debatable if there is indeed a causal relationship between these allergic diseases. Eczema in infancy has been shown to increase the risk of asthma while early onset and severe eczema increases the risk for food allergy (36, 71). A systematic review and meta-analyses on 66 studies, found 16 studies supporting the association between food allergy and severe atopic dermatitis and six indicating association between early

onset atopic dermatitis and food allergy (36). Based on the population-based studies included in the systematic review, up to 15% of children with atopic dermatitis also had food allergy. However, the systematic review did not address differences, if any, between different ancestry backgrounds.

Supporting the causal role of eczema in the development of food allergy is the dual allergen hypothesis described earlier in Chapter 1. The dual allergen hypothesis demonstrates how a defective skin barrier function may have an effect on food allergy. Genetically, loss-of-function mutations in *FLG*, have been shown to be associated with atopic dermatitis (143, 210, 290, 342, 343) and food allergy (123, 126, 213, 214). *FLG* encodes a key epidermal barrier protein and mutations in this gene disrupt the skin barrier (344), enhancing epicutaneous allergen exposure and resulting in an increased transepidermal water loss (TEWL). Interestingly, when comparing patients with eczema who are carrying *FLG* mutations, versus those without *FLG* mutations, increased TEWL was observed in both groups even in presence of non-lesional skin (345). In another study, TEWL was found to be associated with food sensitisation in the absence of eczema (346). These two studies imply that skin barrier impairment may be a precursor to skin lesions and/or inflammation and that skin barrier impairments not specific to atopic dermatitis can also contribute to sensitisation and possibly, food allergy development (345, 346). The impairment and/or permeability of the skin barrier can be affected by infant washing practices in terms of frequency and types of soap and detergents used or even water hardness and might also affect skin barrier permeability (347). Further, it has recently been reported that children (0 to 17 years) with atopic dermatitis and food allergy have a different skin endotype, reflected by an immature skin barrier, than those with eczema alone (348).

Additionally, the frequency and type of *FLG* mutations differs between Asians and Caucasians. Polymorphisms accounting for eczema in Singapore is more diverse compared to that for eczema in Ireland (143). The top eight most frequent *FLG*-null mutations found in the Singapore population account for 80% of *FLG* mutations associated with eczema. In comparison, only two *FLG*-null mutations accounted for 80% of mutations in Ireland. While only three *FLG* mutations, R501X, 2282del4, and E2422X, were common in both European and Asian populations, the most common mutation in the Asian population,

3321delA, was not present in the European population (349). The rare and diverse polymorphisms present in the Asian population make investigating *FLG* in this population problematic.

Of significance is also prevailing evidence for differences in the skin immune response as well as epidermal morphology and dermal composition between Asians and Caucasians (74, 75, 350). In a group of females aged between 18 and 45, East Asians and Caucasians were reported to have higher baseline levels of TEWL compared to African Americans, indicating that the latter have a more intact skin barrier (74). Current literature on TEWL comparisons among different ethnicities have been inconsistent on this note, with some reporting that Caucasians had higher TEWL than African Americans (351). Regardless, the baseline TEWL of Caucasians was slightly higher than East Asians. Despite this, Caucasian skin was shown to have a significantly stronger barrier than East Asian skin, measured by the number of tape stripping required to disrupt barrier (74). These findings are still directly applicable to infants as several studies have shown that the epidermis and stratum corneum, which is important for barrier function from early life (352, 353), of infants are thinner than adults (354-356).

Additionally, East Asian skin has also been reported to be more sensitive and reactive to irritants and environmental agents (357), possibly due to the thinner stratum corneum of East Asian skin (355, 356, 358). Together, this evidence provide further support to the theory that the weaker barrier function in East Asians may be causing the high prevalence of atopic dermatitis in this group increasing their susceptibility to food allergy.

Evidently, there is a scant studies of challenge-proven food allergy in East Asian population. This thesis adds to current knowledge on the trajectory of food allergy progression in early childhood, particularly for children of East Asian-born parents. These findings highlight the children of East Asian-born parents as a high risk allergic group. This group of children therefore might benefit from more frequent monitoring clinically. In particular, findings from this thesis will help better inform clinicians who are caring for children with food allergy and eczema. These findings are also important clinically with regards to targeted implementation of nutritional strategies as a prevention and management strategy. It may be that timely introduction of allergenic food into a child's diet is of paramount importance in children with East Asian-born parents who have atopic dermatitis.

The interplay between atopic eczema, timing of introduction of allergenic food and environmental versus oral exposure may all be crucial in ameliorating development of food allergy.

8.3 Implications of results – genetic aspect

In this thesis, *HLA* and *STAT5B* associations with food allergy were only identified in the Caucasian population living in Australia. While there is some evidence of association between variants of *CTLA4* and egg allergy in the East Asian population living in Australia, the effect sizes were small and associations were weak after correction for multiple testing. These findings warrant additional replication and validation studies to improve our understanding of the genetic contribution of food allergy in the East Asian population and inform clinical practice.

One of the major challenges of identifying unique genetic factors for food allergy is its co-existence with other allergic diseases such as eczema, allergic rhinitis and asthma. Untangling genetic determinants specific to food allergy thus becomes difficult given that there are shared genetic components among the allergic diseases. Several studies have highlighted genes common to allergic diseases (41, 42, 359). These shared candidate genes primarily play a role in the adaptive and innate immune response and skin barrier dysfunction (359). Adding to the complexity is the concept that there are other genetic factors such as ethnicity/ancestry that can also influence the development of these allergic diseases (359).

Although this thesis did not explore potential effects of gene-environment interaction in association with food allergy, this thesis has assessed genes relevant to the East Asian population living in a western environment. The work carried out within the genetic component of this thesis adds to our current understanding of the contribution of genetic risk factors in food allergy among children of East Asian background. Gene-environment interaction studies can be a potential aspect for future research. If shown to mediate the effect of food allergy in the East Asian population, it may be necessary to monitor the East Asian population residing in Asia for evidence of increase in food allergy prevalence in response to changes in environmental factors such as increasing “Westernisation”.

8.4 Strengths and limitations

The studies included in this thesis are nested within the larger HealthNuts study. As part of the HealthNuts study, one of the strengths of this thesis is the use of objective measures such as OFCs and SPT results to diagnose food allergy. While double-blinded placebo controlled OFC is the gold standard (155), we carried out an open OFC without placebo. Open OFCs have been shown to be sufficient for diagnosing objective symptoms in children (9, 10) and are unlikely to cause false-positive results related to subjective symptoms that may arise due to anxiety of undergoing an OFC (155). The OFCs in our study were also carried out on a subgroup of non-sensitised, non-allergic infants to confirm their non-food allergic status. Such objective measures ascertain food allergy status with certainty as opposed to ascertainment based on SPT or specific IgE levels alone. Consequently, the robust phenotyping measures allow specific investigation of differences between sensitisation and allergic status within a group of food sensitised individuals.

Several drawbacks inherent in this thesis have to be taken into account. The most important limitation lies in the small Asian group within the HealthNuts study. This is compounded by the low number of participants who consented to the use of their DNA samples. The genetic component of this thesis also lacked a replication cohort in which we could validate our initial findings. Results derived from this study should therefore be interpreted with caution until these effects are replicated in an independent and larger sample group. Additionally, due to the small sample size of the study population, this thesis has opted for a candidate gene approach instead of a GWAS approach in unravelling the genetic risk factors for food allergy. A limitation to such an approach is the possibility of not capturing and genotyping causal and/or novel variants since genotyped genes were selected based on *a priori* hypothesis.

Another limitation is the definition used for determining Asian ethnicity/ancestry where parental country of birth was used as a proxy of ethnicity. This measure was used in the epidemiological analyses (Chapters 3 and 4) as not all samples were available for genetic ancestry to be inferred. Despite this, a previous study carried out in a subset of the HealthNuts sample showed that parental country of birth is a good and reliable proxy based on an AIMS panel showing congruency between self-reported ethnicity and genetically inferred ancestry (131). Nonetheless, due to a small sample size, we additionally carried

out analyses on all Asian countries together without addressing specific Asian subgroups. In doing so, genetic results obtained from such analyses may be diluted given that a degree of heterogeneity still exists among Asians.

Data collection at 12 months was also done retrospectively, increasing the potential of recall and outcome bias. However, questionnaires were completed prior to knowledge of food allergy status. Therefore, it is unlikely that this would lead to bias in the results by food allergy status. Despite its limitations, the work carried out within this thesis can act as a springboard to develop future research in the field to better understand the differential risk of food allergy observed in different population groups.

8.5 Future directions

Given that food allergy is a multifactorial disease, the complete mechanism behind this disease cannot be explained simply by genetic or environmental factors alone. In identifying genetic risk factors, accounting for a gene-environment interaction might increase the power to detect genes with small marginal effect (48). A gene-environment interaction may help uncover if exposure to certain environmental risk factors can modify risk of food allergy in genetically predisposed infants. An interaction is said to exist when the effect of both genetic and environmental factors on the disease risk are dependent and influenced by one another (50).

Studying the interplay of genes and environment will provide valuable insights into the biological mechanisms on pathogenesis of food allergy. This will help identify a high-risk group that can be targeted and closely monitored for effective treatment and management of food allergy. It is also possible to identify changes in risk of modifiable environmental factors through gene–environment interaction (360). Novel susceptibility genes that are identified will also help provide biomarkers for preventing food allergy through targeted therapeutic interventions in the future. Despite its benefits, it should be noted that interaction studies can require a significant increase in sample size to achieve a level of statistical power sufficient to detect a main effect (360).

It is also imperative to carry out functional epigenetics and gene expression studies to uncover functionality of genetic variants, especially those in non-coding regions, and characterise the biology of the observed associations. Both genetic and environmental

effects may be mediated through intermediate events, such as changes in gene expression or epigenetic processes such as DNA methylation (360). It was shown in a DNA methylation study carried out in neonates that 75% of variably methylated regions were accounted for by gene-environment interactions whereas the remaining 25% were attributed to genetics alone (361). This lends support to the concept of DNA methylation as a mediator of gene-environment interactions. Further, the identified genetic variants associated with food allergy may only reflect the effect of environmental exposures but may not be clinically significant with regards to influencing the risk of developing food allergy (47). It has been suggested that epigenetics may play a role in the differentiation and cytokine gene expression of a sub-group of lymphocytes known as T helper cells. This is of significance as both processes have been identified as important pathways for the development of food allergy (128). Overall, such functional studies will help connect the mechanisms at the molecular level with epidemiological findings at the population level.

Beyond the analytical aspects of the current body of work that can be expanded on, a more significant future direction of this thesis is the formulation of appropriately design multigenerational and/or migration studies. Both multigenerational and migration studies may help elucidate and untangle the mechanisms behind the observed phenomenon of increased food allergy in East Asian migrants in Melbourne, which appears to occur within a single generation. There is increasing evidence from both human and animal model studies that transmission of epigenetic information across generations can occur. This would therefore indicate that intergenerational and transgenerational inheritance can contribute to the risk of allergic diseases. If indeed true that transgenerational and intergenerational effects are implicated in food allergy, it would be essential to establish the clinical relevance of such epigenetic signatures (362). Data obtained from multigenerational studies would add new knowledge and provide a basis to develop novel strategies for interventions (363). It may be that prevention of allergies in the next generation should commence during pregnancy.

In terms of potential migration studies, there are significant challenges and difficulties in designing and implementing such studies. An ideal study design will be one that captures prospective data comprising of robust exposure measures and objectively measured outcomes in the migrant population. The study will ideally be carried out prior to migration

to be able to track any changes in outcomes or exposures with increasing duration of residence in the new country of residence (52). Additionally, the use of serial age-matched controls from both the country of origin and the new country of residence will present a robust group to compare to. Evidently, fulfilling these criteria in a single study is logistically demanding and ambitious. However, addressing several if not all components of these criteria in future studies, will ensure that we are taking the essential steps in the right direction, to uncover underlying mechanisms and risk factors contributing to the stark difference in food allergy prevalence between East Asian and Caucasian children.

8.6 Conclusions

With an increasing population of Asian migrants into Australia, research into the health conditions of this population group has never been timelier. Collectively, this thesis has identified individual environmental and genetic risk factors for food allergy in this population and highlighted the critical importance of interaction between genetic risk factors and the environment. I have shown within this thesis that children with food allergy and eczema at age 1 year have an increased risk of asthma and allergic rhinitis at age 6 years. Prevalence of aeroallergen sensitisation and eczema at age 6 years were higher in children with East Asian-born parents than those with Caucasian-born parents. When comparing children of East Asian-born parents living in Australia to those living in Singapore, the prevalence of food allergy and eczema were higher in Australia. Timely introduction of food which has been shown to be a protective factor for food allergy in Australia, was not as important in Singapore. Despite late introduction of peanut and egg into the diet in children living in Singapore, the prevalence of food allergy in the country remained low.

Given the multifactorial nature of food allergy, it is unlikely that the risk of food allergy is accounted for by environmental factors alone. In this thesis, there is some evidence of association between a reduction in risk of egg allergy and *CTLA4* among egg sensitised East Asian infants. Findings from this thesis also confirmed previous GWAS findings for an association between increased risk of peanut allergy and rs7192 of *HLA* in Caucasian population. Variant rs16967593 of *STAT5B* was also associated with a reduced risk of food allergy in Caucasian infants. There is still a lack of genetic studies in food allergy,

although several GWAS have emerged in recent years. Existing genetic studies were varied in quality, making replication challenging. There is still much room for advancement in the field with the potential of discovering heritable components of food allergy.

Additionally, there is some evidence that susceptibility to food allergy is enhanced in genetically predisposed individuals upon exposure to environmental risk factors. In that vein, the study of interaction between genes and environment on food allergy, is a key area to develop and enhance understanding in aetiology, pathogenesis and progression of food allergy. Nevertheless, further functional, replication and larger studies are required to validate the findings presented here and to establish a causal relationship. It is hoped that this thesis will generate the ideas and pave the way for future studies in food allergy within this population group, with the aim of advancing towards a more targeted and tailored approach to ameliorating food allergy.

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Appendices

Appendix 1 HealthNuts questionnaire age 1 year

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 ☐

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 ☐

18. 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)*	☐	☐	☐

☐

Murdoch Childrens Research Institute

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 Q.29

25. Did you use medication to treat it?

- Never ☐
- Used in the past ☐
- Used now ☐
- Not sure ☐

26. Creams or moisturizers?

No ☐ Yes ☐ Not sure ☐

Name _____

27. Topical Steroids (e.g. cortisone)

No ☐ Yes ☐ Not sure ☐

Name _____

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

No ☐ Yes ☐ Not sure ☐

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 ☐
- Very good ☐
- Good ☐
- Fair ☐
- Poor ☐

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 ☐

→ If no go to Q.44

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 ☐

→ If no go to Q.45

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 ☐

→ If no go to Q.46

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 ☐

→ If no go to Q.47

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 <u>mother</u> 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)	☐	☐	☐

QUESTIONS ABOUT YOUR FAMILY

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

No ☐ Yes ☐

	Date of birth
Brother 1	D D / M M / Y Y Y Y
Brother 2	D D / M M / Y Y Y Y
Sister 1	D D / M M / Y Y Y Y
Sister 2	D D / M M / Y Y Y Y
other siblings	D D / M M / Y Y Y Y

50. Mother's date of birth

D D / M M / Y Y Y Y

51. Father's date of birth

D D / M M / Y Y Y Y

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

- Father ☐
- Mother ☐
- 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 Q.59

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

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

Appendix 2 HealthNuts non-responder questionnaire

Record relevant answer for potential participants that decline to participate:

1) Why have you chosen not to participate in the study?
    - Too many needles
    - Too busy
    - Already eaten the foods
    - NESB
    - Existing food allergy diagnosis
    - Other

2) Has your child tolerated peanuts in ***his/her*** diet?
    - Eaten peanut butter and tolerated
    - Eaten another form of peanut and tolerated
    - Eaten peanut not tolerated
    - Not eaten peanut in any form

3) Does anyone in your immediate family have food allergies?
    - One-year-old
    - Mother
    - Father
    - Sister 1
    - Sister 2
    - Brother 1
    - Brother 2
    - Other sister
    - Other brother

4) Has your one year old child ever been diagnosed with eczema? Y N

5) Does anyone in your immediate family have atopic diseases such as dermatitis, eczema, allergies, hay fever or asthma?
    - Mother
    - Father

- Sister 1
- Sister 2
- Brother 1
- Brother 2
- Other sister
- Other brother

6) Is this your first child?

- Yes
- No, second
- No, third
- No, fourth
- No, other N =

7) What is your postcode? _ _ _ _

NB: questionnaire is completed by researchers i.e. not self-administered

Appendix 3 HealthNuts questionnaire age 6 years

The Royal Children's Hospital Melbourne

Health Nuts — Nuts About Health

Murdoch Childrens Research Institute — Healthier Kids. Healthier Future.

HealthNuts Questionnaire

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: Postcode: State:

Phone: Mobile: Home:( )

Work:( ) Email:

For *child's* father (or guardian)

Given name(s): Surname:

Address – Number and Street:

Address – Suburb: Postcode: State:

Phone: Mobile: Home:( )

Work:( ) 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 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?

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
1.1 Peanut butter	☐ 1	☐ 2	☐ 3	☐ 4
1.2 Peanuts	☐ 1	☐ 2	☐ 3	☐ 4
1.3 Pistachios	☐ 1	☐ 2	☐ 3	☐ 4
1.4 Cashews	☐ 1	☐ 2	☐ 3	☐ 4
1.5 Almonds	☐ 1	☐ 2	☐ 3	☐ 4
1.6 Hazelnuts (including Nutella)	☐ 1	☐ 2	☐ 3	☐ 4
1.7 Pine nuts	☐ 1	☐ 2	☐ 3	☐ 4
1.8 Other nuts *(please specify)*	☐ 1	☐ 2	☐ 3	☐ 4
1.9 Tahini (or hummus)	☐ 1	☐ 2	☐ 3	☐ 4
1.10 Sesame seeds on foods (e.g. bread, sesame snaps)	☐ 1	☐ 2	☐ 3	☐ 4
1.11 Semi-cooked (runny) egg (e.g. scrambled, soft boiled, fried, poached)	☐ 1	☐ 2	☐ 3	☐ 4
1.12 Completely cooked (hard) egg (e.g. hard boiled, fried, poached)	☐ 1	☐ 2	☐ 3	☐ 4
1.13 Meringue, pavlova or macaroons	☐ 1	☐ 2	☐ 3	☐ 4
1.14 Cakes containing egg	☐ 1	☐ 2	☐ 3	☐ 4
1.15 Biscuits containing egg (e.g. teddy bear biscuits)	☐ 1	☐ 2	☐ 3	☐ 4
1.16 Other foods containing egg *(please specify)*	☐ 1	☐ 2	☐ 3	☐ 4
1.17 Fish	☐ 1	☐ 2	☐ 3	☐ 4
1.18 Shellfish *(please specify)*	☐ 1	☐ 2	☐ 3	☐ 4
1.19 Soy milk or other soy products *(please specify)*	☐ 1	☐ 2	☐ 3	☐ 4
1.20 Cow's milk (including on cereal)	☐ 1	☐ 2	☐ 3	☐ 4
1.21 Cow's milk in baked products (e.g. cakes, muffins)	☐ 1	☐ 2	☐ 3	☐ 4
1.22 Other dairy products (e.g. cheese, yoghurt, cream, ice cream) *(please specify)*	☐ 1	☐ 2	☐ 3	☐ 4
1.23 Other types of milk (e.g. goat's milk) *(please specify)*	☐ 1	☐ 2	☐ 3	☐ 4
1.24 Wheat (e.g. bread, cakes, biscuits)	☐ 1	☐ 2	☐ 3	☐ 4

1.25 Do you restrict any particular foods in your child's diet? ☐ 1 No ☐ 2 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 if you 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)	☐ 1	☐ 2	☐ 3
b)	Seafood (including fish)	☐ 1	☐ 2	☐ 3
c)	Oily fish (e.g. salmon, fresh tuna, trout, mackerel, sardines)	☐ 1	☐ 2	☐ 3
d)	Fruit	☐ 1	☐ 2	☐ 3
e)	Vegetables (green and root)	☐ 1	☐ 2	☐ 3
f)	Pulses (peas, beans, lentils)	☐ 1	☐ 2	☐ 3
g)	Cereal (including bread)	☐ 1	☐ 2	☐ 3
h)	Pasta	☐ 1	☐ 2	☐ 3
i)	Rice	☐ 1	☐ 2	☐ 3
j)	Butter	☐ 1	☐ 2	☐ 3
k)	Margarine	☐ 1	☐ 2	☐ 3
l)	Nuts	☐ 1	☐ 2	☐ 3
m)	Potatoes	☐ 1	☐ 2	☐ 3
n)	Cow's milk *Please specify type (e.g. full fat, low fat etc)*	☐ 1	☐ 2	☐ 3
o)	Other types of milk *Please specify type (e.g. soy, almond etc)*	☐ 1	☐ 2	☐ 3
p)	Eggs	☐ 1	☐ 2	☐ 3
q)	Fast food / takeaway	☐ 1	☐ 2	☐ 3
r)	Soft drink	☐ 1	☐ 2	☐ 3
s)	Cordial	☐ 1	☐ 2	☐ 3
t)	Fruit juice	☐ 1	☐ 2	☐ 3
u)	Powdered nutritional supplements *(e.g. Sustagen)*	☐ 1	☐ 2	☐ 3

1.27 **Has your child ever been fed toddler formula or follow-on formula (e.g. Karicare Toddler or Heinz Nurture Follow-on)?**

☐ 1 No → **go to Question 2.1**

☐ 2 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**?

☐ 1 No ---→ **go to Question 2.2** ☐ 2 Yes *(answer questions below)….*

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: peanut	**Years** Y 5	**Months** M 3	☑	☐	☑	☐	☐	☑	redness on face	☑	☐	☐
a	Y Y	M M	☐	☐	☐	☐	☐	☐		☐ 1	☐ 2	☐ 3
b	Y Y	M M	☐	☐	☐	☐	☐	☐		☐ 1	☐ 2	☐ 3
c	Y Y	M M	☐	☐	☐	☐	☐	☐		☐ 1	☐ 2	☐ 3
d	Y Y	M M	☐	☐	☐	☐	☐	☐		☐ 1	☐ 2	☐ 3
e	Y Y	M M	☐	☐	☐	☐	☐	☐		☐ 1	☐ 2	☐ 3
f	Y Y	M M	☐	☐	☐	☐	☐	☐		☐ 1	☐ 2	☐ 3
g	Y Y	M M	☐	☐	☐	☐	☐	☐		☐ 1	☐ 2	☐ 3

2.2 Since age 4, have you 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**? ☐$_1$ No ☐$_2$ Yes

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?

☐$_1$ No ---→ go to Question 3.2 ☐$_2$ Yes ☐$_3$ Don't know

If yes...

a. Has your child had this itchy rash at any time in the last 12 months?
☐$_1$ No ☐$_2$ Yes ☐$_3$ 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?
☐$_1$ No ☐$_2$ Yes ☐$_3$ Don't know

c. At what age did this itchy rash first occur?
☐$_1$ Under 2 years ☐$_2$ Age 2-4 years ☐$_3$ Age 5-6 years

d. Has this rash cleared completely at any time in the last 12 months?
☐$_1$ No ☐$_2$ Yes ☐$_3$ 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?
☐$_1$ Never in the last 12 months
☐$_2$ Less than one night per week
☐$_3$ One or more nights per week

3.2 Has your child been diagnosed with eczema?

☐$_1$ No ☐$_2$ Yes ---→ a. Age when symptoms started ☐ year(s) **or** ☐ months old

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

☐ 1 No ☐ 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)

☐ Never had eczema, itchy rash or dry skin ---→ go to Question 3.5

☐ Had/have eczema, itchy rash or dry skin but never used ---→ go to Question 3.5

☐ Used in the past

☐ Use now

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

a. Have you used moisturisers?

☐ 1 No ☐ 2 Yes Name ☐ 3 Don't know

b. Have you used topical steroid creams or ointments (e.g. sigmacort, celestone, elocon, cortic, hydrocortisone, advantan fatty ointment)?

☐ 1 No ☐ 2 Yes Name ☐ 3 Don't know

c. Did you use steroid creams for more than 10 days in a row?

☐ 1 No ☐ 2 Yes ☐ 3 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?

☐ 1 None

☐ 2 1 to 5

☐ 3 6 to 10

☐ 4 More than 10

3.6 Since age 4, have you 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.	Dermatologist	☐ How many times did you see a dermatologist?	
e.	Other health professional *(please specify)*	☐ How many times did you see this health professional?	
f.	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?**

☐ 1 No ---→ go to Question 3.8 ☐ 2 Yes ☐ 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?

☐ 1 No ---→ go to Question 3.8 ☐ 2 Yes

c. How many attacks of wheezing has your child had in the last 12 months?

☐ 1 None ☐ 2 1 to 3 ☐ 3 4 to 12 ☐ 4 More than 12

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

☐ 1 Never woken with wheezing
☐ 2 Less than one night per week
☐ 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?

☐ 1 No ☐ 2 Yes

3.8 **Has your child ever had asthma?**

☐ 1 No
☐ 2 Yes ---→ a) Were you told by a doctor that your child had asthma? ☐ 1 No ☐ 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?

☐ 1 No ☐ 2 Yes ☐ 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?**

☐ 1 No ---→ go to Question 3.10 ☐ 2 Yes ☐ 3 Not sure

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

	When wheezy	Regularly (every day for at least 2 months)
a. Name of 'Western' medicine	*(tick one box for each line)*	
☐	☐ 1	☐ 2
☐	☐ 1	☐ 2
☐	☐ 1	☐ 2
b. Name of 'Alternative' medicine		
☐	☐ 1	☐ 2
☐	☐ 1	☐ 2

3.10 **Since age 4, have you 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?	

3.11 **In the past 12 MONTHS, how many times has your child been admitted to the hospital because of wheezing or asthma?**

☐ 1 None	☐ 4 3 to 5
☐ 2 1	☐ 5 6 to 10
☐ 3 2	☐ 6 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?**

☐ 1 None	☐ 3 6 to 10
☐ 2 1 to 5	☐ 4 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?**

☐ 1 No ---→ **go to Question 3.14** ☐ 2 Yes ☐ 3 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?

☐ 1 No ---→ **go to Question 3.14** ☐ 2 Yes ☐ 3 Don't know

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

☐ 1 No ☐ 2 Yes ☐ 3 Don't know

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

☐ 1 Not at all ☐ 2 A little ☐ 3 A moderate amount ☐ 4 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

3.14 Has your child ever had hay fever?

☐ 1 No ---→ go to Question 3.16

☐ 2 Yes ---→ a. Were you told by a doctor that your child had hay fever? ☐ 1 No ☐ 2 Yes

b. Age when symptoms started ☐☐ year(s) old

3.15 Since age 4, have you consulted any health professionals about your child's hay fever?

No ☐ ---→ If no, go to Question 3.16

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?	☐☐

3.16 In the past **12 MONTHS**, how much did any nose problem interfere with your child's daily activities?

☐ 1 Not at all
☐ 2 A little
☐ 3 A moderate amount
☐ 4 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?

☐ 1 No ☐ 2 Yes ☐ 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?

☐ 1 None
☐ 2 1 to 5
☐ 3 6 to 10
☐ 4 More than 10

4. OTHER QUESTIONS ABOUT YOUR CHILD

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

☐ 1 Excellent ☐ 2 Very good ☐ 3 Good ☐ 4 Fair ☐ 5 Poor

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

☐ 1 No ---→ go to Question 4.3

☐ 2 Yes ---→ a. How old was he/she when they first had worms?

☐ 1 Less than 1 year ☐ 2 1 to 3 years ☐ 3 4 to 6 years

b. How many times has he/she 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	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b.	on weekends	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
	Winter					
c.	on weekdays	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d.	on weekends	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

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 any brothers or sisters (including half siblings)?

☐ 1 No ---→ go to Question 4.7 ☐ 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 ½ the time in the child's household?
1	D D / M M / Y Y	☐ 1 Sister ☐ 2 Brother	☐ 1 Full ☐ 3 Half, father common ☐ 2 Half, mother common ☐ 4 Other	☐ 1 No ☐ 2 Yes
2	D D / M M / Y Y	☐ 1 Sister ☐ 2 Brother	☐ 1 Full ☐ 3 Half, father common ☐ 2 Half, mother common ☐ 4 Other	☐ 1 No ☐ 2 Yes
3	D D / M M / Y Y	☐ 1 Sister ☐ 2 Brother	☐ 1 Full ☐ 3 Half, father common ☐ 2 Half, mother common ☐ 4 Other	☐ 1 No ☐ 2 Yes
4	D D / M M / Y Y	☐ 1 Sister ☐ 2 Brother	☐ 1 Full ☐ 3 Half, father common ☐ 2 Half, mother common ☐ 4 Other	☐ 1 No ☐ 2 Yes
5	D D / M M / Y Y	☐ 1 Sister ☐ 2 Brother	☐ 1 Full ☐ 3 Half, father common ☐ 2 Half, mother common ☐ 4 Other	☐ 1 No ☐ 2 Yes
6	D D / M M / Y Y	☐ 1 Sister ☐ 2 Brother	☐ 1 Full ☐ 3 Half, father common ☐ 2 Half, mother common ☐ 4 Other	☐ 1 No ☐ 2 Yes

Please list any other siblings and answer questions above:

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 <u>past ONE month</u>.**

		Never	Almost Never	Some-times	Often	Almost Always
	<u>Physical Functioning (problems with…)</u>					
a.	Walking more than one block	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b.	Running	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c.	Participating in sports activity or exercise	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d.	Lifting something heavy	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e.	Taking a bath or shower by him or herself	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f.	Doing chores, like picking up his or her toys	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
g.	Having hurts or aches	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
h.	Low energy level	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
	<u>Emotional Functioning (problems with...)</u>					
i.	Feeling afraid or scared	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
j.	Feeling sad or blue	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
k.	Feeling angry	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
l.	Trouble sleeping	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
m.	Worrying about what will happen to him or her	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
	<u>Social Functioning (problems with…)</u>					
n.	Getting along with other children	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
o.	Other kids not wanting to be his or her friend	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
p.	Getting teased by other children	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
q.	Not able to do things that other children his or her age can do	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
r.	Keeping up when playing with other children	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
	<u>School Functioning (problems with...)</u>					
s.	Pay attention in class	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
t.	Forgetting things	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
u.	Keeping up with school activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
v.	Missing school because of not feeling well	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
w.	Missing school to go to doctor or hospital	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

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	☐ 1	☐ 2	☐ 3
b.	Restless, overactive, cannot stay still for long	☐ 1	☐ 2	☐ 3
c.	Often complains of headaches, stomach-aches or sickness	☐ 1	☐ 2	☐ 3
d.	Shares readily with other children, for example toys, treats, pencils	☐ 1	☐ 2	☐ 3
e.	Often loses temper	☐ 1	☐ 2	☐ 3
f.	Rather solitary, prefers to play alone	☐ 1	☐ 2	☐ 3
g.	Generally well behaved, usually does what adults request	☐ 1	☐ 2	☐ 3
h.	Many worries or often seems worried	☐ 1	☐ 2	☐ 3
i.	Helpful if someone is hurt, upset or feeling ill	☐ 1	☐ 2	☐ 3
j.	Constantly fidgeting or squirming	☐ 1	☐ 2	☐ 3
k.	Has at least one good friend	☐ 1	☐ 2	☐ 3
l.	Often fights with other children or bullies them	☐ 1	☐ 2	☐ 3
m.	Often unhappy, depressed or tearful	☐ 1	☐ 2	☐ 3
n.	Generally liked by other children	☐ 1	☐ 2	☐ 3
o.	Easily distracted, concentration wanders	☐ 1	☐ 2	☐ 3
p.	Nervous or clingy in new situations, easily loses confidence	☐ 1	☐ 2	☐ 3
q.	Kind to younger children	☐ 1	☐ 2	☐ 3
r.	Often lies or cheats	☐ 1	☐ 2	☐ 3
s.	Picked on or bullied by other children	☐ 1	☐ 2	☐ 3
t.	Often volunteers to help others (parents, teachers, other children)	☐ 1	☐ 2	☐ 3
u.	Thinks things out before acting	☐ 1	☐ 2	☐ 3
v.	Steals from home, school or elsewhere	☐ 1	☐ 2	☐ 3
w.	Gets along better with adults than with other children	☐ 1	☐ 2	☐ 3
x.	Many fears, easily scared	☐ 1	☐ 2	☐ 3
y.	Good attention span, sees tasks through to the end	☐ 1	☐ 2	☐ 3

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 <u>new symptoms or diagnoses</u> of .....

		No one	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 *(specify insect)*	☐	☐	☐	☐	☐	☐	☐	☐
f.	Food allergy *(specify food)*	☐	☐	☐	☐	☐	☐	☐	☐

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

☐ 1 No ---→ go to Question 5.4 ☐ 2 Yes

If yes,

			Inside and outside	Outside only	Inside only
a.	Number of cats:	☐	☐ 1	☐ 2	☐ 3
b.	Number of dogs:	☐	☐ 1	☐ 2	☐ 3
c.	Number of birds:	☐	☐ 1	☐ 2	☐ 3
d.	Other pet *(please specify)*	☐	☐ 1	☐ 2	☐ 3

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

☐ 1 No ☐ 2 Yes, avoid cats only ☐ 3 Yes, avoid dogs only ☐ 4 Yes, avoid both cats and dogs

☐ 5 Yes, avoids other pets *(please specify)*

5.4 Do you live on a farm or property with any animals (livestock)? ☐ 1 No ☐ 2 Yes

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?

☐1 Never ☐2 Sometimes ☐3 Usually

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

	Mother	Father
Year 10 or less	☐1	☐2
Year 11	☐1	☐2
Year 12	☐1	☐2
Trade apprenticeship	☐1	☐2
Technical diploma/certificate	☐1	☐2
University degree	☐1	☐2
Postgraduate university degree	☐1	☐2
Other *(please specify)*	☐1	☐2

5.10 a. Is the child's **mother** currently in paid work? ☐1 No ☐2 Part-time ☐3 Full-time

b. Is the child's **father** currently in paid work? ☐1 No ☐2 Part-time ☐3 Full-time

5.11 a. Child's **mother's** current weight
(if currently or recently pregnant, record pre-pregnancy weight): ☐☐☐.☐ kg

b. Child's mother's height: ☐☐☐ cm

c. Child's mother's date of birth: D D / M M / Y Y

5.12 a. Child's **father's** current weight: ☐☐☐.☐ kg

b. Child's father's height: ☐☐☐ cm

c. Child's father's date of birth: D D / M M / Y Y

5.13 In which country/region was your child born?

☐1 Australia / New Zealand	☐6 America
☐2 Europe	☐7 Asia (e.g. Singapore, Malaysia, Vietnam)
☐3 India / Pakistan / Bangladesh	☐8 Middle East (e.g. Egypt, Syria)
☐4 Africa	☐9 Other ☐
☐5 United Kingdom	

5.14 Child's **mother's** ethnicity?

- ☐ 1 Caucasian
- ☐ 2 Asian
- ☐ 3 African
- ☐ 4 Aboriginal or Torres Strait Islander
- ☐ 5 Middle Eastern
- ☐ 6 Other *(please specify)*

5.15 Child's **father's** ethnicity?

- ☐ 1 Caucasian
- ☐ 2 Asian
- ☐ 3 African
- ☐ 4 Aboriginal or Torres Strait Islander
- ☐ 5 Middle Eastern
- ☐ 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)*
☐ 1 Caucasian	☐ 1 Caucasian	☐ 1 Caucasian	☐ 1 Caucasian
☐ 2 Asian	☐ 2 Asian	☐ 2 Asian	☐ 2 Asian
☐ 3 African	☐ 3 African	☐ 3 African	☐ 3 African
☐ 4 Aboriginal or Torres Strait Islander	☐ 4 Aboriginal or Torres Strait Islander	☐ 4 Aboriginal or Torres Strait Islander	☐ 4 Aboriginal or Torres Strait Islander
☐ 5 Middle Eastern	☐ 5 Middle Eastern	☐ 5 Middle Eastern	☐ 5 Middle Eastern
☐ 6 Other *(please specify)*	☐ 6 Other *(please specify)*	☐ 6 Other *(please specify)*	☐ 6 Other *(please specify)*

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 aunt/uncle) we could contact?

Given name(s):

Surname:

Relationship to the child:

Address – Number and Street:

Address – Suburb: Postcode: State:

Phone: Mobile: Home: ( )

Email:

Appendix 4 Protocol for separation of peripheral blood mononuclear cells, plasma and granulocytes from whole blood

Materials and solutions

2 mL Cryobank vials (NUNC Intermed, Roskilde, Denmark)

2 mL Cryogenic vials (Corning Inc., NY, USA)

10 x Boyles solution (200 mL contains 16.2 g NH_4CL, 2.0 g $KHCO_3$)

Freezing container, Mr Frosty (Nalgene, NY, USA)

Trypan blue exclusion dye (Sigma, MO, USA)

Transport Medium - Gibco® RPMI-1640 medium (Invitrogen, CA, USA)

10 IU/mL preservative free heparin in RPMI-1640 Medium

Heat-inactivated Fetal Calf Serum (HI-FCS) (Life technologies, Australia)

Place 50 mL sterile Fetal Calf Serum in a water-bath at 56^{o}C for 30 minutes. Aliquot to 10 mL volumes and store at 4^{o}C until required.

RPMI-1640 Medium containing 2% (v/v) Heat-inactivated FCS (RPMI/HI-FCS)

For all cell washes, RPMI/HI-FCS is used and must be at room temperature before use.

Approximately 50 mL is needed for every blood sample to be processed. Add 1 mL of HI-FCS solution to 50 mL of RPMI-1640.

Ficoll-Paque (Lymphoprep) Tubes (Axis-Shield PoC AS, Oslo, Norway)

Add 2.5 mL Ficoll-Paque (Lymphoprep) solution to every 10 mL tube. For every 10 mL of blood collected, two Lymphoprep tubes are required. Make sure these are at room temperature before use.

Freezing Mix (HI-FCS containing 15% (v/v) dimethyl sulfoxide, DMSO (Sigma-Aldrich, USA))

Add 7.5 mL of DMSO to 42.5 mL of HI-FCS. This needs to be done on ice and the DMSO added slowly (i.e. dropwise) to the HI-FCS to avoid heating the sample. The Freezing Mix once prepared is stored at 4^{o}C.

Protocol

Blood must be at room temperature and separation commenced within 2 hours of collection PBMC isolation to be done using aseptic technique. Samples must be kept at room temperature and all media must be brought to room temperature until cryopreservation

Separation of Plasma:

1. Centrifuge blood tubes at 700 *g* for 10 minutes (brake on) and collect plasma in a fresh 10 mL tube.
2. Plasma is centrifuged again at 700 *g* for 10 minutes (brake on) to remove platelets and then aliquoted into 1 mL cryovials and stored at -80^{o}C.

Separation of PBMCs:

1. Fill blood tube to 10 mL with Transport medium (once in Transport medium, PBMCs must be separated within 18 hours. Leave at room temperature during this time).
2. Layer blood onto fresh, sterile 10 mL tubes containing 2.5 mL of Ficoll-Paque (or Lymphoprep). For 10 mL of blood, require 2 Ficoll-Paque tubes. Using a sterile transfer pipette, tilt the Ficoll-Paque tube sideways and slowly pipette blood dropwise down the side of the tube and onto the surface of the Ficoll-Paque solution. Cap the tube to maintain sterility.
3. Centrifuge tubes at 500 *g* for 30 minutes at room temperature with the brake off.
4. Carefully aspirate the mononuclear cells at the interface between the transport medium and Ficoll-Paque solution using a sterile transfer pipette and pool into new sterile 10 mL tube. If volume of mononuclear cells is > 5 mL, use 2 x 10 mL tubes. Fill tube with RPMI containing 2% (v/v) heat-inactivated Fetal Calf Serum (RPMI/HI-FCS) and invert to mix. The RPMI/HI-FCS must be at room temperature prior to use (NB:

each tube must contain at least an equal volume of RPMI/HI-FCS solution and mononuclear cells).

5. Centrifuge at 500 *g* for 10 minutes at room temperature (brake on) and discard supernatant.
6. Wash cells carefully by resuspending cell pellet with 1mL RPMI/HI-FCS solution using a sterile transfer pipette and once fully resuspended, fill tube with RPMI/HI-FCS.
7. Centrifuge at 500 *g* for 7 minutes at room temperature (brake on) and discard supernatant.
8. Repeat Step 7 and resuspend final cell pellet with 1mL RPMI/HI-FCS and perform cell count.
9. Mix equal volume (10 µL) of cell suspension and Trypan blue exclusion dye (for cell viability) into a single well of a 96-well microtitre plate and load 10 µL onto a Haemocytometer and count cells.

Counting Cells

1. Use the inner squares on the haemocytometer and count at least one row of squares (ie. 5 squares or ~200 cells). Use the following formula to count the total number of cells:

Cryopreservation of Cells

1. Cell suspension (1 mL) must be kept on ice at all times during this procedure.
2. Place Freezing mix on ice and slowly add 1 mL dropwise down the side of the cell suspension tube over a period of 1 minute. NB: Gently mix solution on ice during this process as this an exothermic reaction
3. Total volume should now be 2 mL (should be 1:1 ratio of Freezing mix and RPMI/HI-FCS).
4. Cryovials should contain 8-10 x 106 PBMCs/mL. Aliquot 1 mL cell suspension into each cryovial as necessary and place into a freezing container (Mr Frosty or equivalent; rate-freezing: -1^{o}C/minute).
5. Store at -80^{o}C for at least 4 hours. Do not move/disturb container during this time.
6. Transfer cryovials to permanent liquid nitrogen storage within 24 hours.

Granulocyte isolation using Red blood cell lysis:

1. Place into a fresh 50 mL falcon tube the RBC fraction from Leucosep tube
2. Fill the 50 mL tubes with 1x Boyles solution.
3. Mix by inversion and leave in room temperature for 5min (with frequent inversion).
4. Red blood cell lysis has occurred when the tube turns from opaque to transparent red. Leave the tubes no longer than an extra 5 minutes
5. Spin at 805 *g* (2000 RPM) for 10 minutes
6. Remove supernatant
7. Add PBS to cells to resuspend. Then add PBS to 30 mL to rehydrate cells. Leave at room temperature for at least 15 minutes.
8. Spin down cells at 350 *g* for 10 minutes
9. Remove Supernatant
10. Prepare Freeze mix (Fetal Calf Serum + 10% DMSO)
11. Add 2 mL of freezing media per sample, resuspend and store as 2 x 1 mL aliquots
12. Transfer cells into Mr Frosty after 15 – 30 minutes contact time with DMSO and leave over night at -80^{o}C.

Appendix 5 Published manuscript for Chapter 3

Original Article

Children with East Asian-Born Parents Have an Increased Risk of Allergy but May Not Have More Asthma in Early Childhood

Noor H.A. Suaini, BSc (Hons)[a,b], Jennifer J. Koplin, PhD[b,c], Rachel L. Peters, PhD[a,b], Mari Sasaki, MD[b], Justine A. Ellis, PhD[a,d,e], David J. Martino, PhD[a,b,f], Adrian J. Lowe, PhD[b,c], Mimi L.K. Tang, MBBS, FRACP, FRCPA, PhD[a,b,g], Anne-Louise Ponsonby, BMedSci, MBBS, PhD[b,c], Lyle C. Gurrin, PhD[c], Shyamali C. Dharmage, MBBS, MSc, MD, PhD[c], and Katrina J. Allen, MBBS, FRACP, PhD, FAHMS[a,b,g,h]; for the HealthNuts study group *Parkville, Carlton, and Burwood, Victoria, Australia; and Manchester, United Kingdom*

What is already known about this topic? Infants with East Asian-born parents living in Melbourne have a higher risk of IgE-mediated food allergy than those with Caucasian-born parents. It is unclear if this increased risk extends to other allergic diseases later in childhood.

What does this article add to our knowledge? IgE-mediated food allergy and eczema at age 1 increase the risk of asthma and allergic rhinitis at age 6, but this association was not modified by ancestry. Children with East Asian-born parents do go on to have more allergic diseases at age 6 years, whereas atopic asthma appears to be similar to children with Caucasian-born parents.

How does this study impact current management guidelines? Clinicians will be able to use these data to better inform parents of East Asian children on the progression of their child's allergic diseases early in their childhood. Future prevention and management approaches to allergic diseases can be tailor-made and targeted to this population.

BACKGROUND: We previously reported that infants with Asian-born parents are 3 times more likely to have IgE-mediated food allergy than those with Australian-born parents. It is unknown whether this translates to the increased risk of other allergic diseases later in childhood and whether ancestry interacts with other risk factors for allergic disease development.

OBJECTIVE: To compare prevalence and risk factors for allergic rhinitis, asthma, and aeroallergen sensitization at age 6 between children with East Asian-born and Caucasian-born parents.

METHODS: A total of 5276 1-year-old infants were recruited into a population-based longitudinal study of allergy. A total of 4455 children participated in age 6 follow-up (84.4%), including 3015 with Caucasian-born parents and 415 with East Asian-born parents. Children underwent skin prick tests to aeroallergens and questionnaires captured data on asthma, eczema, and allergic rhinitis.

RESULTS: Compared with children with Caucasian-born parents, children of East Asian-born parents had more allergic rhinitis (19.9% [95% confidence interval (CI) 14.9-26] vs 9.3% [95% CI 8-10.8], $P < .001$) and aeroallergen sensitization (64.3% [95% CI 57.5-70.5] vs 34.4% [95% CI 32.2-36.7], $P < .001$) at age 6. Asthma was similar in both groups (9.1%

[a]Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia
[b]Centre for Food and Allergy Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia
[c]The School of Population and Global Health, University of Melbourne, Carlton, Victoria, Australia
[d]Centre for Social and Early Emotional Development, Faculty of Health, Deakin University, Burwood, Victoria, Australia
[e]Genes, Environment & Complex Disease, Murdoch Children's Research Institute, Parkville, Victoria, Australia
[f]Telethon Kids Institute, University of Western Australia, Perth, Western Australia, Australia
[g]Department of Allergy and Clinical Immunology, Royal Children's Hospital, Parkville, Victoria, Australia
[h]Institute of Inflammation and Repair, University of Manchester, Manchester, United Kingdom

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Conflicts of interest: M. L. K. Tang is on the Nestle Nutrition Institute Medical Advisory Board Oceania; is a past member of the Danone Nutricia Global Scientific Advisory Board; has received consultancy fees from Deerfield Consulting, GLG Consulting, and Bayer; is employed by and has stock/stock options in ProTA Therapeutics; has received lecture fees from Danone Nutricia and Nestle Health Sciences; has a patent owned by Murdoch Children's Research Institute; received royalties from Wilkinson Publishing; and has received payment for developing educational presentations from MD Linx. K. J. Allen serves as a consultant for Nestle, ThermoFisher, AspenCare, Before Brands, and Nutricia. The rest of the authors declare that they have no relevant conflicts of interest.

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Corresponding author: Katrina J. Allen, MBBS, FRACP, PhD, FAHMS, Murdoch Children's Research Institute, Royal Children's Hospital, Flemington Road, Parkville 3052, Victoria, Australia. E-mail: katrina.allen@mcri.edu.au.

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Abbreviations used
CI- Confidence interval
ISAAC- International Study of Asthma and Allergies in Childhood
OFC- Oral food challenge
PR- Prevalence ratio
SACC- Standard Australian Classification of Countries
SPT- Skin prick test

[95% CI 6.2-13.2] vs 11.7% [95% CI 10.4-13.1]), $P = .21$. Children with IgE-mediated food allergy and eczema in infancy were 3 times more likely to have asthma and 2 times more likely to have allergic rhinitis at age 6, irrespective of ancestry.

CONCLUSIONS: Children of East Asian ancestry born in Australia have a higher burden of most allergic diseases in the first 6 years of life, whereas asthma may follow a different pattern. IgE-mediated food allergy and eczema at age 1 increase the risk of asthma and allergic rhinitis irrespective of ancestry. **(J Allergy Clin Immunol Pract 2019;7:539-47)**

Key words: *Allergic rhinitis; Ancestry; Asians; Asthma; Children; Eczema; Ethnicity; Food allergy; Hay fever*

There is increasing evidence that the risk of allergic disease differs according to both ancestry and the environment in early life. We previously showed that children of Asian descent who were born in Australia had very high rates of eczema and food allergy in early life,[1,2] with up to 50% of infants with both parents born in East Asia having eczema and 25% having challenge-confirmed food allergy by 1 year of age.[3] In contrast, children who were born in Asia and subsequently migrated to Australia in early childhood appeared to be protected from developing food allergy.[1] This difference in allergy prevalence by both ancestry and country of birth is not limited to Australia. A study in the United States found that children born outside the United States had a lower risk of food sensitization than those born in the United States, whereas the highest risk of food sensitization was seen among children born in the United States to migrant parents.[4] Another study in the United Kingdom found an over-representation of non-Caucasian children in a pediatric allergy clinic.[5]

Despite the amplified burden of allergic disease in infancy among children of Asian ancestry who are born in Australia, little is known about their risk of allergic disease later in life. In general, it has been estimated that of those with eczema during the first 4 years of life, around one-third progress to develop asthma and two-thirds develop allergic rhinitis.[6-8] It is also generally accepted that early life food allergy and eczema clinically coassociate.[9] Less is known about the role of food allergy in the development of the atopic march to asthma and allergic rhinitis, although we have previously reported that children with food allergy at age 1 year were more likely to have a doctor diagnosis of asthma at 4 years of age and that the risk was highest for children with food allergy and coexistent eczema in infancy.[10] It is not known whether the atopic march is modified by ancestry or country of birth.

As such, the aims of this study were: (1) to compare the prevalence of allergic rhinitis, asthma, and aeroallergen sensitization in Australian children with East Asian-born and Caucasian-born parents at age 6 years and (2) to investigate whether the associations between IgE-mediated food allergy and eczema in the first year of life and allergic disease at age 6 years differ depending on ancestry, using data from our recently completed age 6 follow-up of the HealthNuts population-based longitudinal study of allergic disease.

METHODS

Study population

The HealthNuts study is a longitudinal population-based cohort study of allergic disease in Melbourne, Australia. The recruitment process has previously been described in detail.[11] Briefly, 5276 twelve-month-old infants were recruited from immunization clinics around Melbourne (74% participation rate). All infants underwent a skin prick test (SPT) at recruitment to 4 foods (egg, peanut, sesame, shrimp/cow's milk) (ALK-Abello, Madrid, Spain). Infants with a detectable wheal size $\geq$1 mm to any of the foods were invited to the HealthNuts clinic at the Royal Children's Hospital, Melbourne, for an oral food challenge (OFC) to ascertain their food allergy status. OFCs were carried out using a predetermined protocol as previously described.[11,12]

Age 6-year follow-up. At 6 years of age, all children (N = 5276) were invited to attend a HealthNuts allergy clinic at the Royal Children's Hospital. Home visit assessments were offered for those who were unable to attend the hospital. Parents completed a questionnaire that included general information about the child as well as family and the child's history of asthma, wheeze, allergic rhinitis, and eczema. We also incorporated questions on asthma, eczema, and allergic rhinitis from the validated International Study of Asthma and Allergies in Childhood (ISAAC).[13] Those who did not complete the full questionnaire were given the option to complete a short telephone questionnaire, which asked a limited number of questions including whether the child had ever been diagnosed with asthma or allergic rhinitis.

Skin prick tests. At age 6 years, all children who participated in an assessment, either at the Royal Children's Hospital or home visit, underwent a SPT to peanut, egg, sesame, soy, almond, cashew, hazelnut, shellfish, cow's milk, wheat, house dust mite, rye grass, Bermuda grass, cat hair, alternaria, birch mix, cladosporium, and dog hair. Aeroallergen sensitizations were determined in the whole cohort only at age 6. SPTs were carried out with a single-tine lancet (Stallergenes, Antony, France) on the child's back using allergen extracts (ALK-Abello) including a positive control (10 mg/mL histamine) and a 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 and then subtracting the negative control SPT diameter.

Definitions

Ancestry. Parental country of birth was used as a proxy for ancestry background. We have shown previously that parental country of birth information correlated well with genetically inferred ancestry (93.7% correlation for "Caucasians" and 93.0% correlation for "Asians").[14] For this analysis, we focused on 2 groups, East Asians and Caucasians. Caucasians refer to children with both parents being born in Australia, the United Kingdom, or Europe. The East Asian group was made up of children with 1 or 2 East Asian-born parents, as defined in one of our previous studies.[3] Our East Asian definition included countries in the North East Asia region such as China,

Hong Kong, Japan, Taiwan, Macau, North Korea, South Korea, and South East Asia region of Vietnam, Philippines, Singapore, Thailand, Indonesia, Malaysia, Cambodia, and Laos. Groupings were based on the Standard Australian Classification of Countries (SACC) that were developed to be relevant to Australia's multicultural society for use in analyzing Australian-based country of origin data.[15] Groups in the SACC comprise geographically proximate countries that have broadly similar social, cultural, economic, and political characteristics. All other country groups (eg, South Asians, Middle East, and Africa) were not included in the analyses due to small numbers. We also correlated parental country of birth with self-reported grandparents' ancestry. Eighty-seven percent of children classified in the East Asian group had at least 1 set of grandparents reporting Asian as their ancestry, whereas 80% of those in the Caucasian group had both sets of grandparents reporting as Caucasian.

Definitions for allergy at age 1 year

Food allergy. Food allergy is defined as a positive OFC outcome or recent reaction consistent with our OFC stopping criteria[12] to peanut, sesame, or egg, in conjunction with a positive sensitization test (SPT with wheal size $\geq$ 2 mm greater than negative control and/or sIgE $>$ 0.35 kU/L). A positive OFC was defined as more than 3 noncontact urticarial reactions lasting more than 5 minutes, angioedema, vomiting, or anaphylaxis, within 2 hours of the last challenge dose. On discharge, those with a negative challenge (able to tolerate top dose of challenged food without any subsequent allergic reactions) were administered a single serving of the challenged food at home for 7 days to capture any late reactions.

Infantile eczema. Infantile eczema is defined as parent report doctor diagnosis of eczema during the first year of life.

Definitions for allergy at age 6

Eczema. Parent report of an itchy rash in the last 12 months that affected typical eczema locations, such as folds of elbows and knees.[16]

Aeroallergen sensitization. Positive SPT wheal $\geq$3 mm greater than negative control for any of the following aeroallergens: house dust mite, rye grass, Bermuda grass, cat hair, alternaria, birch mix, cladosporium, and dog hair.

Allergic rhinitis. Allergic rhinitis was defined as nose symptoms in the last 12 months accompanied by itchy watery eyes[13,16] in the presence of aeroallergen sensitization.

Asthma. Asthma was defined as parent report of a doctor diagnosis of asthma and either wheeze or use of asthma medication in the last 12 months.

Atopic asthma. We also further classified asthma into atopic asthma (asthma with aeroallergen sensitization) and nonatopic asthma (asthma with no aeroallergen sensitization).[17,18]

Statistical analysis

This is a *post hoc* cohort analysis, as a follow-up to our previous study that found children with East Asian-born parents have an increased risk of food allergy at age 1 year.[3]

Prevalence estimates. The prevalence of allergy outcomes at age 6 years in each ancestry group was estimated as the observed proportion with 95% confidence intervals (CIs) generated using the normal approximation to the binomial distribution. To control for the potential impact of differential loss to follow-up, we adjusted for differences in demographic characteristics and other potential risk factors between participants who completed the full questionnaire at age 6 years and those who were lost to follow-up/not included in the analysis, using the inverse probability weighting method described by Little and Rubin[19] (see Online Repository at www.jaci-inpractice.org for details).

Regression models. For each ancestry, the association between allergy status at age 1 (food allergy and infantile eczema) and risk of asthma and allergic rhinitis at age 6 was estimated using binomial regressions (a generalized linear model with a logarithm link function) to obtain estimates of prevalence ratios (PRs) and 95% CIs for the corresponding population parameter. Interaction analyses between ancestry and food allergy and eczema at age 1 year were tested by adding product terms to the regression model.

Wheezing in the first year of life could be an early indicator of asthma.[20] Therefore, to investigate the longitudinal association between food allergy at age 1 year and the subsequent development of asthma, that is, to assess newly incident disease after age 1, we repeated our analyses after excluding children with wheezing in the first year of life.

The regression analyses were adjusted for the following potential confounders based on previous published literature: sex, socioeconomic status, and parent's or sibling's history of asthma, eczema, allergic rhinitis, or food allergy. Additional potential confounders considered include the presence of cat or dog in the household, household smoking, maternal smoking during pregnancy, mode of delivery, antibiotic use in the first year of life, birthweight ($<$2500 g vs $\geq$2500 g), duration of breastfeeding, number of siblings, and season of birth (winter vs other).[21,22] These were included in the regression models if they changed the magnitude of the association between the exposure and outcome by more than 10% on the PR scale.

Sensitivity analyses. We performed the following sensitivity analyses for our regression models, to examine the potential impact of loss to follow-up on our results, and to examine whether our findings were robust to different definitions of asthma and allergic rhinitis:

(1) To control for the potential impact of differential loss to follow-up, we adjusted for differences in demographic characteristics and other potential risk factors between participants who completed the full questionnaire at age 6 years and those who were lost to follow-up/not included in the analysis, using the inverse probability weighting method described by Little and Rubin[19] (see Online Repository at www.jaci-inpractice.org for details).
(2) To reduce the amount of missing data at age 6 years, we combined data on asthma and allergic rhinitis diagnosis from the full and short questionnaire, and repeated the analysis to examine consistency of our results while using data from a greater percentage of the original cohort.
(3) To examine whether our findings were robust when using different definitions of asthma and allergic rhinitis, we repeated the analyses using the ISAAC definitions of asthma (answering Yes to both questions "Has your child ever had wheezing or whistling in the chest at any time in the past?" and "Has your child had wheezing in the past 12 months?") and allergic rhinitis (answering Yes to both questions "Has your child ever had sneezing or runny or blocked nose when he/she did not have a

TABLE I. Demographics and baseline characteristics of study population included in main analyses that included children with full questionnaire only

Characteristic	Caucasian (n = 2620)	East Asian (n = 352)	*P* value
Demographics			
Birthweight, g (mean, range)	3451 (630-5160)	3208 (692-4690)	.032
Male	1323 (50.7)	204 (58.6)	.005
Infant's season of birth			
Summer	597 (22.8)	77 (21.9)	.319
Autumn	638 (24.4)	75 (21.3)	
Winter	701 (26.8)	93 (26.4)	
Spring	683 (26.1)	107 (30.4)	
Caesarean delivery	863 (33)	110 (31.3)	.53
≤ 36 wk of gestation	144 (5.7)	23 (7)	.354
Quintiles of SEIFA disadvantage			
1 (most disadvantaged)	451 (17.2)	53 (15.1)	.416
2	538 (20.6)	84 (23.9)	
3	577 (22.1)	79 (22.4)	
4	548 (21)	64 (18.2)	
5 (least disadvantaged)	501 (19.2)	72 (20.5)	
Family history of asthma, eczema, allergic rhinitis, or food allergy	1956 (74.7)	244 (69.3)	.032
Environmental exposure			
No. of siblings			
No siblings	1306 (50.3)	189 (54)	.213
1 sibling	864 (33.2)	116 (33.1)	
2 siblings	337 (13)	32 (9.1)	
3 or more siblings	92 (3.5)	13 (3.7)	
Use of antibiotics	1293 (51)	150 (44.5)	.026
Childcare attendance	741 (28.3)	78 (22.2)	.016
Cat ownership	514 (19.6)	21 (6)	<.001
Dog ownership	976 (37.3)	52 (14.8)	<.001
Household smoking	504 (19.3)	65 (18.5)	.703
Maternal smoking during pregnancy	105 (4)	2 (0.6)	.001
Infant's diet			
Any breastfeeding	2498 (95.8)	341 (97.2)	.233
Exclusive breastfeeding	1398 (61.3)	148 (47.9)	<.001
Any formula feeding	1856 (76.3)	285 (84.6)	.001

SEIFA, Socio-Economic Indices for Areas.

cold or flu?" and "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/flu?").[13]

The results of each sensitivity analysis are reported in the Online Repository at www.jaci-inpractice.org.

All analyses were performed using Stata 15 for Windows (StataCorp LP, College Station, Tex). Venn diagrams were obtained using Venn Diagram Plotter (PNNL, Richland, Wash).[23] The Venn Diagram Plotter is supported by the W.R. Wiley Environmental Molecular Science Laboratory, a national scientific user facility sponsored by the US Department of Energy's Office of Biological and Environmental Research and located at PNNL. PNNL is operated by Battelle Memorial Institute for the US Department of Energy under contract DE-AC05-76RL0 1830.

Ethics approval

Ethics approval was obtained from the Royal Children's Hospital Human Research Ethics Committee (reference nos. 27047 and 32294).

RESULTS

Study population

Of the 5276 infants recruited at 12 months, 84% participated in the age 6 follow-up, with a majority answering the full questionnaire (n = 3663). An additional 605 participants completed the short questionnaires and were included in our sensitivity analyses (see Figure E1, available in this article's Online Repository at www.jaci-inpractice.org). Assessments including SPT were completed by 3233 children at age 6 years. Demographic and baseline characteristics of the study population are provided in Table I.

Prevalence of allergic diseases at age 6

Allergic rhinitis and eczema at 6 years of age were more common in East Asian children than Caucasian children (Figure 1). Allergic rhinitis was present in 19.9% (95% CI 14.9-26.0) of East Asian children and 9.3% (95% CI 8.0-10.8) of Caucasian children ($P < .001$). Eczema was present in 26.0% (95% CI 21.0-31.7) of East Asian children

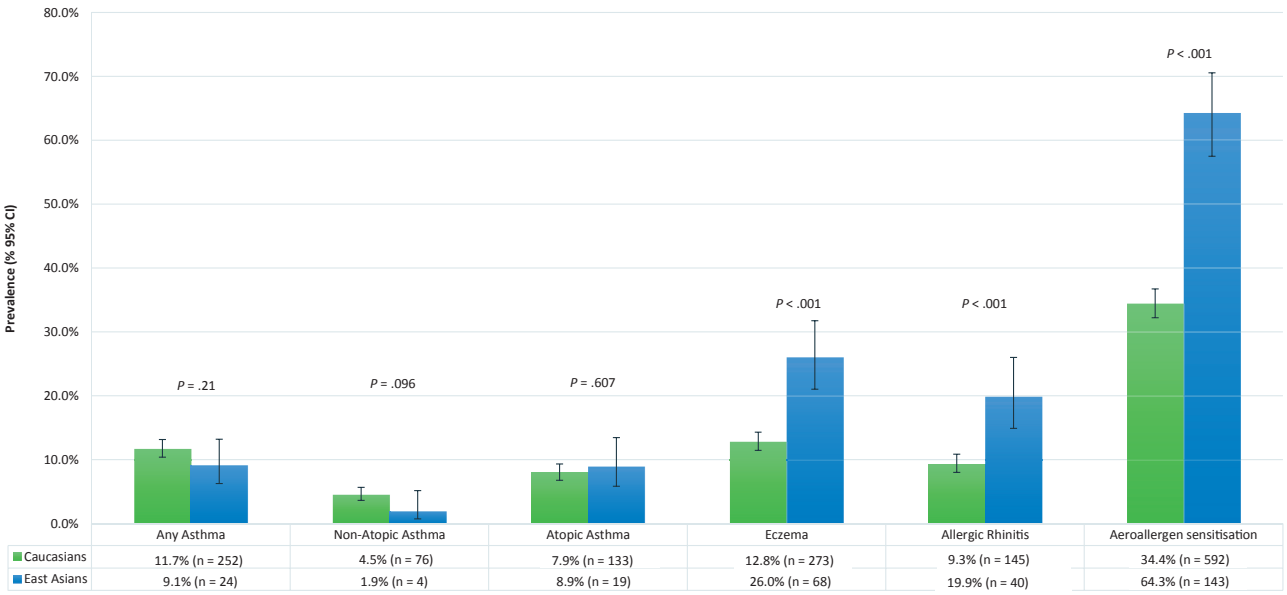

FIGURE 1. Weighted prevalence of asthma, allergic rhinitis, eczema, and atopic asthma at age 6, stratified by ancestry, with 95% confidence interval. Prevalence estimates were adjusted (weighted) for differences in demographic characteristics and other potential risk factors between participants who completed the full questionnaire at age 6 years and those who were lost to follow-up/not included in the analysis, using the inverse probability weighting method described by Little and Rubin[19] (see Online Repository at www.jaci-inpractice.org for details).

and 12.8% (95% CI 11.5-14.3) of Caucasian children ($P < .001$).

The prevalence of aeroallergen sensitization was also higher in East Asian children, with 64.3% (95% CI 57.5-70.5) sensitized to at least 1 aeroallergen compared with 34.4% (95% CI 32.2-36.7) of Caucasian children ($P < .001$). When aeroallergens were examined individually, sensitization to each of the tested allergens was higher in the East Asian children (data not shown).

Conversely, asthma prevalence was similar in East Asian and Caucasian children, affecting 9.1% (95% CI 6.2-13.2) of East Asian children and 11.7% (95% CI 10.4-13.1) of Caucasian children ($P = .21$; Figure 1). When assessed as asthma phenotypes, East Asian children had a lower prevalence of nonatopic asthma (1.9%, 95% CI 0.7-5.1) compared with Caucasian children (4.5%, 95% CI 3.6-5.7) ($P = .096$), whereas the prevalence of atopic asthma was similar in both groups (8.9% vs 7.9%, $P = .607$).

An increase in prevalence of allergic disease was observed when comparing children with 1 versus 2 East Asian-born parents (Figure E2, available in this article's Online Repository at www.jaci-inpractice.org).

Furthermore, among East Asian infants with eczema, food allergy, or both at age 1, 12.8% (95% CI 8.1-19.6) had asthma at age 6, compared with 17.8% (95% CI 15.0-20.9) of Caucasian infants with eczema, food allergy or both at age 1 ($P = .165$).

Relationship between aeroallergen sensitization and asthma

The overlap between aeroallergen sensitization and asthma in Caucasian and East Asian children is shown in Figure 2. Overall, 70% of East Asian children had either aeroallergen sensitization and/or asthma compared with only 41% of Caucasian children. This was due to a high prevalence of aeroallergen sensitization in the East Asian group. However, fewer of the aeroallergen-sensitized East Asian children also had asthma compared with the aeroallergen-sensitized Caucasian children (15% vs 24%, $P = .01$).

Associations between early life eczema and food allergy status and asthma at age 6

Table II shows the relationship between infantile food allergy and eczema and diagnosed asthma at age 6 years. Caucasian children with both eczema and food allergy at age 1 were 3 times as likely to have asthma at age 6 compared with those with no eczema and no food allergy at age 1 (PR 3.56, 95% CI 2.72-4.67, $P < .001$). The magnitude of association was similar in East Asian children (PR 3.12, 95% CI 1.21-8.08, $P = .019$). There was no evidence that the association between food allergy and eczema status at age 1 and asthma at age 6 differed by ancestry. The associations observed in each ancestry group were broadly similar after excluding children with a history of wheeze in the first year of life, although the association seen in children with eczema alone was reduced (Table II).

Caucasian children with food allergy or eczema alone were twice as likely to have asthma at age 6. Food allergy alone remains associated with asthma after excluding Caucasian children with a history of wheeze (Table II). These findings were not observed in East Asian children.

Similar results were observed in a sensitivity analysis that included sampling weights to adjust for differences in those lost to follow-up compared with those who participated at age 6 years (see Table E1, available in this article's Online Repository at www.jaci-inpractice.org), as well as in sensitivity analyses using broader definitions of asthma (see Table E2, available in this article's Online Repository at www.jaci-inpractice.org) and

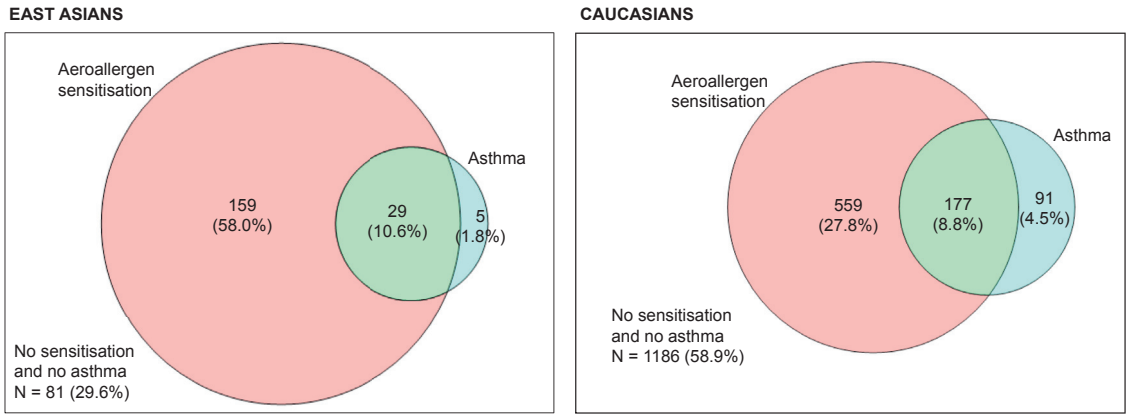

FIGURE 2. Aeroallergen sensitization and asthma at age 6 years in children with East Asian-born parents (n = 274) and Caucasian-born parents (n = 2013).

TABLE II. Association between food allergy and eczema at 1 y with asthma at 6 y of age, stratified by ancestry

	Caucasian (n = 2620)				East Asian (n = 352)				
Variable	**Total**	**Asthma (%)**	**PR* (95% CI)**	***P* value**	**Total**	**Asthma (%)**	**PR* (95% CI)**	***P* value**	***P* interaction**
All infants									
No eczema or food allergy	1662	151 (9.1)	1.0	–	135	8 (6.0)	1.0	–	–
Food allergy only	92	21 (22.8)	2.45 (1.62-3.71)	<.001	24	3 (12.5)	1.89 (0.49-7.37)	.357	.966
Eczema only	464	63 (13.6)	1.46 (1.11-1.92)	.007	71	5 (7.0)	1.14 (0.37-3.49)	.816	.788
Eczema and food allergy	154	52 (33.8)	3.56 (2.72-4.67)	<.001	49	12 (24.5)	3.12 (1.21-8.08)	.019	.874
Excluding infants wheezing during first year of life									
No eczema or food allergy	1276	103 (8.1)	1.0	–	105	8 (7.6)	1.0	–	–
Food allergy only	73	15 (20.6)	2.52 (1.55-4.11)	<.001	20	2 (10)	1.36 (0.28-6.54)	.699	.584
Eczema only	326	34 (10.4)	1.28 (0.89-1.85)	.184	47	0	Omitted†	–	–
Eczema and food allergy	117	37 (31.6)	3.8 (2.74-5.28)	<.001	29	9 (31)	3.5 (1.26-9.69)	.016	.983

CI, Confidence interval.
*Prevalence ratio (PR) adjusted for sex, socioeconomic status, family history of allergic disease, and antibiotics use.
†Prevalence ratio could not be calculated because there were no children with asthma in this group.

ISAAC definitions (see Table E3, available in this article's Online Repository at www.jaci-inpractice.org).

Associations between early life eczema and food allergy status and allergic rhinitis at age 6

Among Caucasian children, those with both eczema and food allergy at age 1 were 4-fold more likely to have allergic rhinitis at age 6 compared with those with no eczema and no food allergy at age 1 (PR 4.26, 95% CI 2.97-6.11). Although the magnitude of this association was lower for Asian children (PR 2.29, 95% CI 1.1-4.8), there was only modest evidence that ancestry modified the association between food allergy and eczema status at age 1 and allergic rhinitis at age 6 (*P* interaction = .091, Table III). Food allergy alone was also associated with allergic rhinitis in both Caucasian and East Asian children, whereas eczema alone was only associated with allergic rhinitis in Caucasian children (Table III).

Similar results were observed in the sensitivity analyses (see Tables E1-E3, available in this article's Online Repository at www.jaci-inpractice.org), although the magnitude of some of the associations was attenuated.

DISCUSSION

We have shown through this study that East Asian children have a higher prevalence of most allergic diseases at 6 years of age. Asthma appears to be similar between East Asian and Caucasian children. However, there was no evidence of a differential progression of the atopic march in East Asian children compared with Caucasian children. Children with both IgE-mediated food allergy and eczema during infancy had an increased risk of allergic rhinitis and asthma at age 6, regardless of ancestry. Caucasian children with food allergy or eczema alone also had an increased risk of asthma and allergic rhinitis, although the magnitude of association was lower than those with both food allergy and eczema.

Our finding that asthma appears to follow a different pattern to other allergic diseases in East Asian children was unexpected. Sensitization to aeroallergens has been shown to be a strong risk factor for wheezing[24] and asthma.[25] However, although aeroallergen sensitization was twice as common in East Asian children, the prevalence of asthma was not similarly increased. Asthma was less common in East Asian children with aeroallergen sensitization compared with aeroallergen sensitized children of Caucasian parents. A previous study of 3 South East

TABLE III. Association between food allergy and eczema at 1 y with allergic rhinitis at 6 y of age, stratified by ancestry

	Caucasian (n = 2620)				East Asian (n = 352)				
Variable	Total	Allergic rhinitis (%)	PR* (95% CI)	*P* value	Total	Allergic rhinitis (%)	PR* (95% CI)	*P* value	*P* interaction
No eczema or food allergy	1190	76 (6.4)	1.0	—	95	11 (11.6)	1.0	—	—
Food allergy only	69	13 (18.8)	2.81 (1.67-4.74)	<.001	19	8 (42.1)	4.3 (2.19-8.42)	<.001	.688
Eczema only	333	51 (15.3)	2.16 (1.54-3.04)	<.001	55	13 (23.6)	1.64 (0.75-3.56)	.213	.559
Eczema and food allergy	114	34 (29.8)	4.26 (2.97-6.11)	<.001	41	13 (31.7)	2.29 (1.1-4.8)	.027	.091

CI, Confidence interval.
*Prevalence ratio (PR) adjusted for sex, socioeconomic status, and family history of allergic disease.

Asian populations also reported that the prevalence of asthma did not correspond well to the prevalence of atopy in these populations—despite differences in the prevalence of asthma between Hong Kong (7%), Kota Kinabalu, Malaysia (3%), and San Bu, China (2%), the prevalence of atopy (defined by positive SPT) in these countries was similar.[26] In the same study, Leung and Ho also found that family history was a stronger risk factor for asthma and allergic disease than aeroallergen sensitization in these 3 populations.

There are several possible explanations for the finding that asthma, unlike other allergic diseases, was not more common among East Asian children. Ethnic differences in lung function have been well documented across all ages.[27-31] Previous studies reported that, compared with Caucasians, South and North East Asians had reduced forced expired volume in 1 second (a measure of airway caliber) and forced vital capacity (a measure of lung size),[27] citing inspiratory muscle strength, lung compliance, or chest size as possible explanations for the differences.[29,32,33] Lack of power to detect a difference is another potential issue, given that the prevalence of asthma was lower than other allergic diseases. Nevertheless, the marked differences observed between the 2 ancestry groups in terms of sensitization rates suggest that this is not the sole reason for the findings. In addition, nondifferential misclassification (such as misclassification of viral wheeze as asthma) could reduce the magnitude of the association. Finally, it is possible that East Asian parents are less likely to recognize or seek medical diagnosis of asthma symptoms in their child. However, this latter possibility seems unlikely because the East Asian group was more often diagnosed with other allergic conditions, showing that this group was being seen in the health system. The reasons for this finding warrant further exploration.

Apart from asthma, other allergic diseases (IgE-mediated food allergy, eczema, aeroallergen sensitization, and allergic rhinitis) were very common in East Asian children. It has been proposed that the historical parasite endemic of the previous era shaped the evolution of the immune system in populations residing in tropical regions such as those in East Asia.[34] Genetic studies have shown correlations between geographical parasite prevalence and genetic diversity in genes involved in immune defense, and immune disease.[35] These signatures of genetic adaptation in the immune system point to regional endemic pathogen load as a strong selective pressure on human evolution. Theoretically, the selection of particular proinflammatory genotypes under historically high pathogen load may predispose to an "over-active" immune profile in modern environments, where many chronic infections have been eliminated and migration is common. This might contribute to the extremely high rates of allergy in East Asian children born into the westernized environment of Australia. It has also been shown that age at migration is an important determinant for prescription of asthma medication, suggesting that environmental exposure in early life is critical.[36]

Our findings add to current knowledge about the role of infant food allergy, primarily, IgE-mediated food allergy, separate from and in conjunction with eczema, in the atopic march. Previous studies have predominantly focused on eczema or food sensitization in early life, and studies of challenge-proven food allergy are limited.[37,38] In addition, no previous studies have explored potential differences in the atopic march in children with different ethnic backgrounds. We also found several demographic differences, such as gender, pet ownership, and childcare attendance between Caucasians and East Asians, which may have contributed to differences in allergic disease prevalence between these groups. We have adjusted for these factors in our logistic regression models by including them as confounders if the magnitude of association between exposure and outcome changed by more than 10%.

One of the strengths of this study cohort is the high participation rate both at recruitment and at follow-up. Recruited participants are also broadly representative of the general population.[11] Furthermore, most food allergy studies are based on self-reported food allergy and an advantage of our study is the use of OFC to confirm food allergy status. Notwithstanding, our study does have some limitations. We found differences in several demographic and other characteristics for those lost to follow-up compared with those who participated in follow-up at age 6 years. To account for this participation bias, we adjusted for these differences using reweighting, which showed that our results remained consistent. It should also be noted that diagnosis of wheeze/asthma remains challenging at age 6. It is likely that some children may have continuing early transient wheeze or infectious induced symptoms. Atopic asthma, which generally becomes persistent, only occurred in a minority of patients (<5%). Moreover, the overall prevalence of asthma was low, which limits the likelihood of finding any association to ethnicity. Therefore, the negative finding for asthma requires further investigation. The follow-up of this cohort at age 10 years is currently underway and may shed more light on the issue. Our categorization for East Asians and Caucasians was based on parental country of birth that was used as a proxy for ancestry. Although some misclassification is likely using this definition, we have supporting evidence from our own study that self-reported ancestry correlates well with genetically inferred ancestry in this population.[14] In addition, we have grouped all the East Asian countries together to increase statistical power, but there might be some differences between countries that we were unable to explore. We were also not powered to look at other Asian groups

such as the South Asians. Lastly, the lack of difference in association between food allergy and eczema status at age 1 and asthma diagnosis or allergic rhinitis at age 6 by ancestry may be due to the small sample size of East Asian children in the study.

CONCLUSIONS

High rates of allergy among East Asian children in infancy appear to be maintained into early childhood, with a high prevalence of eczema and allergic rhinitis at 6 years of age. Atopic asthma appears to be similar between East Asian and Caucasian children. Our findings identify East Asian children as a high-risk allergic group not just in infancy but throughout early childhood. We also showed that IgE-mediated food allergy and eczema in infancy increase the risk of asthma and allergic rhinitis in early childhood, irrespective of ancestry.

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ONLINE REPOSITORY

SENSITIVITY ANALYSIS TO EXPLORE THE POTENTIAL IMPACT OF MISSING DATA

We have previously described differences in the characteristics of participants who completed the full questionnaire, the short questionnaire, and nonparticipants at age 4 years.[E1] To mitigate potential bias in estimated prevalence ratios due to differential participation in follow-up, we generated sampling weights that were used to adjust (via reweighting) for differences in these factors between participants with and without missing data at age 6 years, using the inverse probability weighting method described by Little and Rubin.[E2] Weights were calculated as the inverse of the predicted probability of inclusion at age 6 years, from a logistic regression model of participation including as covariates risk factors that were associated with completion of the full questionnaire rather than the short questionnaire or nonparticipation (the child's socioeconomic status, family history of allergy, parents' country of birth, whether or not the child had a challenge-confirmed food allergy at age 1 year, childcare attendance, infant formula use, dog ownership, maternal smoking during pregnancy, number of siblings, and child's eczema diagnosis at age 1 year). Essentially, we calculated a propensity score for each participant,[E3] and these weights were included in the logistic regression models (see Table E1), with robust standard errors used to ensure that the precisions of estimated risks reflected the sample size.

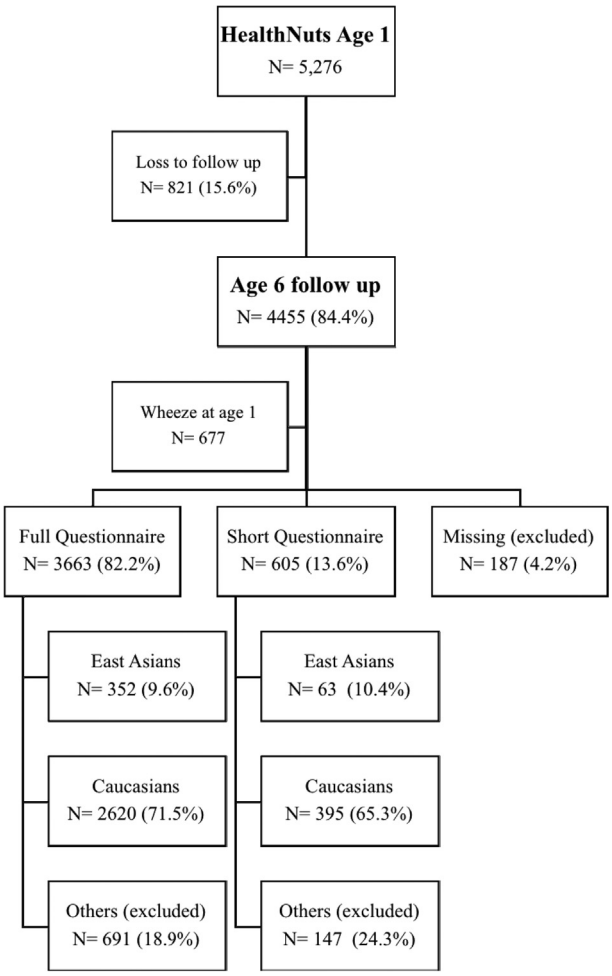

FIGURE E1. Flowchart of age 6 follow-up of the HealthNuts cohort.

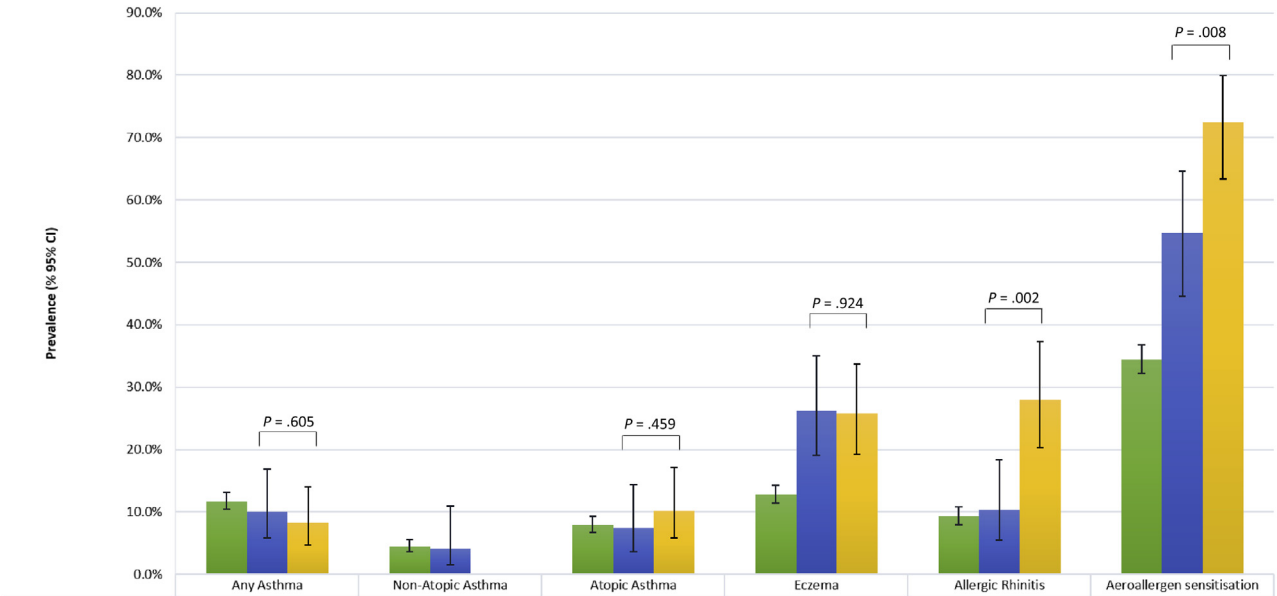

FIGURE E2. Weighted prevalence of allergic diseases, with 95% confidence interval, stratified by parental country of birth. *P* values shown are based on comparison between 1 and 2 East Asian-born parents. No *P* value is given for the nonatopic asthma group as there were no cases of nonatopic asthma among children with 2 East Asian-born parents.

TABLE E1. Sensitivity analysis—association between food allergy and eczema at 1 y and asthma and allergic rhinitis at age 6, with sampling weights included in the model

	Caucasian (n = 2620)		East Asian (n = 352)		
Variable	**PR (95% CI)**	***P* value**	**PR (95% CI)**	***P* value**	***P* interaction**
Asthma*					
No eczema or food allergy	1.0	—	1.0	—	
Food allergy only	2.25 (1.43-3.55)	<.001	2.62 (0.72-9.52)	.143	.788
Eczema only	1.3 (0.96-1.74)	.086	1.41 (0.45-4.38)	.552	.824
Eczema and food allergy	3.32 (2.49-4.43)	<.001	4 (1.52-10.52)	.005	.546
Allergic rhinitis*					
No eczema or food allergy	1.0	—	1.0	—	—
Food allergy only	2.79 (1.55-5.03)	.001	3.06 (1.36-6.9)	.007	.821
Eczema only	2.18 (1.51-3.14)	<.001	1.73 (0.8-3.75)	.166	.637
Eczema and food allergy	4.28 (2.88-6.38)	<.001	2.11 (0.95-4.69)	.068	.132

Factors included in reweighting were socioeconomic status, family history of allergy (eczema, food allergy, allergic rhinitis), parent's country of birth, infant's eczema diagnosis at age 1, infant's egg allergy status at age 1, infant's peanut allergy status at age 1, attendance at childcare, dog ownership, use of infant formula, number of siblings, and maternal smoking during pregnancy.
CI, Confidence interval; *PR*, prevalence ratio.
*Analyses were adjusted for sex.

TABLE E2. Sensitivity analysis—association between food allergy and eczema at age 1 and asthma and allergic rhinitis diagnosis at age 6 from a combined full and short questionnaire

	Caucasian (n = 2620)		East Asian (n = 352)		
Variable	**PR (95% CI)**	***P* value**	**PR (95% CI)**	***P* value**	***P* interaction**
Asthma diagnosis*					
No eczema or food allergy	1.0	–	1.0	–	
Food allergy only	2.05 (1.37-3.05)	<.001	1.65 (0.48-5.65)	.422	.753
Eczema only	1.65 (1.32-2.07)	<.001	1.13 (0.43-2.96)	.801	.403
Eczema and food allergy	3.32 (2.61-4.21)	<.001	3.81 (1.72-8.47)	.001	.697
Allergic rhinitis diagnosis†					
No eczema or food allergy	1.0	–	1.0	–	
Food allergy only	1.91 (0.9-4.03)	.09	1.03 (0.38-2.82)	.953	.364
Eczema only	2.66 (1.91-3.71)	<.001	0.97 (0.47-1.99)	.932	.008
Eczema and food allergy	3.07 (1.92-4.91)	<.001	1.23 (0.6-2.52)	.564	.035

CI, Confidence interval; *PR*, prevalence ratio.
*Adjusted for sex, socioeconomic status, family history of allergic disease, and use of antibiotics.
†Adjusted for sex, socioeconomic status and family history of allergic disease.

TABLE E3. Sensitivity analysis—association between food allergy and eczema at 1 y and ISAAC questions on asthma and allergic rhinitis at age 6

	Caucasian (n = 2620)		East Asian (n = 352)		
Variable	**PR (95% CI)**	***P* value**	**PR (95% CI)**	***P* value**	***P* interaction**
Wheeze*					
No eczema or food allergy	1.0	–	1.0	–	
Food allergy only	1.91 (1.36-2.68)	<.001	4.08 (1.61-10.38)	.003	.245
Eczema only	1.17 (0.94-1.47)	.162	1.52 (0.58-4)	.398	.57
Eczema and food allergy	2.87 (2.33-3.54)	<.001	3.16 (1.34-7.41)	.008	.521
Nose symptoms†					
No eczema or food allergy	1.0	–	1.0	–	
Food allergy only	1.33 (0.94-1.87)	.105	1.82 (1.11-2.99)	.018	.354
Eczema only	1.38 (1.17-1.64)	<.001	1.1 (0.68-1.76)	.698	.25
Eczema and food allergy	2.4 (2.01-2.87)	<.001	1.57 (1-2.46)	.052	.044

CI, Confidence interval; *ISAAC*, International Study of Asthma and Allergies in Childhood; *PR*, prevalence ratio.
*Wheeze was defined as any whistling in the chest or wheezing in the last 12 mo. Analysis was adjusted for sex, socioeconomic status, family history of allergic disease, and use of antibiotics.
†Nose symptoms defined as sneezing or runny/blocked nose when child did not have cold/flu in the last 12 mo. Analysis was adjusted for sex, socioeconomic status, and family history of allergic disease.

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Appendix 6 Published manuscript for Chapter 5

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REVIEW ARTICLE

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Genetic determinants of paediatric food allergy: A systematic review

Noor H. A. Suaini[1,2] | Yichao Wang[1,2] | Victoria X. Soriano[1,2] | David J. Martino[1,2,3] | Katrina J. Allen[1,2,4,5] | Justine A. Ellis[1,6,7] | Jennifer J. Koplin[2,8]

[1]Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia

[2]Centre for Food and Allergy Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[3]Telethon Kids Institute, University of Western Australia, Perth, Western Australia, Australia

[4]Department of Allergy and Clinical Immunology, Royal Children's Hospital, Parkville, Victoria, Australia

[5]Institute of Inflammation and Repair, University of Manchester, Manchester, UK

[6]Genes, Environment & Complex Disease, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[7]Centre for Social and Early Emotional Development, Faculty of Health, Deakin University, Burwood, Victoria, Australia

[8]School of Population and Global Health, University of Melbourne, Parkville, Victoria, Australia

Correspondence
Jennifer J. Koplin, Royal Children's Hospital, Murdoch Children's Research Institute, Parkville, Vic., Australia.
Email: jennifer.koplin@mcri.edu.au

Abstract

Background: The genetic determinants of food allergy have not been systematically reviewed. We therefore systematically reviewed the literature on the genetic basis of food allergy, identifying areas for further investigation.

Methods: We searched three electronic databases (MEDLINE, EMBASE and PubMed) on 9 January 2018. Two authors screened retrieved articles for review according to inclusion criteria and extracted relevant information on study characteristics and measures of association. Eligible studies included those that reported an unaffected nonatopic control group, had genetic information and were carried out in children.

Results: Of the 2088 studies retrieved, 32 met our inclusion criteria. Five were genome-wide association studies, and the remaining were candidate gene studies. Twenty-two of the studies were carried out in a predominantly Caucasian population with the remaining 10 from Asian-specific populations or unspecified ethnicity. We found *FLG*, *HLA*, *IL10*, *IL13*, as well as some evidence for other variants (*SPINK5*, *SERPINB* and *C11orf30*) that are associated with food allergy.

Conclusions: Little genetic research has been carried out in food allergy, with *FLG*, *HLA* and *IL13* being the most reproducible genes for an association with food allergy. Despite promising results, existing genetic studies on food allergy are inundated with issues such as inadequate sample size and absence of multiple testing correction. Few included replication analyses or population stratification measures. Studies addressing these limitations along with functional studies are therefore needed to unravel the mechanisms of action of the identified genes.

KEYWORDS

food allergy, genetics, single nucleotide polymorphisms, systematic review

1 | INTRODUCTION

Food allergy is a complex multifactorial disease with both environmental and genetic risk factors thought to contribute to its pathogenesis. It elicits abnormal immunological reaction upon exposure to certain food proteins, resulting in adverse clinical reactions, most severely anaphylaxis, which can be life-threatening.[1]

Existing twin and family studies have shown that genetic composition may play a significant role in the development of food allergy.[2-4] In these studies, genetic differences contribute about 15%-35% of the observed individual differences in food-specific

Abbreviations: CNVR, Copy number variation region; CNVs, Copy number variations; GWAS, Genome-wide association study; SNP, Single nucleotide polymorphisms; UTR, Untranslated region.

IgE.[4] Twin studies found that monozygotic twins recorded higher concordance rates for sensitization to peanut allergen than dizygotic twins.[2,3] Sicherer et al[3] found that the heritability estimate for peanut allergy was 82%-87%, demonstrating the role of genetic influence as those with more similar genes (monozygotic twins) were likely to have a more similar phenotype.

The prevalence of food allergy in infants and children below 5 years old appears to be higher in Western countries, compared with Asian countries.[5] However, Australian-born children of Asian parents have a higher prevalence of food allergy compared with both Asian children born in Asia and Australian-born Caucasian children.[6,7] This suggests that the effect of genetic predisposition on food allergy may differ depending on environmental exposures in early life.

Both candidate gene and genome-wide association studies (GWAS) have attempted to identify genes associated with food allergy. An increasing number of GWAS are being carried out primarily for "any food allergy" and peanut allergy outcomes, identifying novel genes associated with these allergies. However, these studies were predominantly in Caucasian or European populations. Candidate gene studies have targeted immune-related genes postulated to be involved in the mechanisms of food allergy. Additionally, given that there are shared genetic risk factors among asthma, allergic rhinitis and eczema,[8,9] there has been work to examine genes previously associated with other allergic diseases for an association with food allergy. However, compared to other allergic diseases, the genetic basis of food allergy remains relatively under-explored. The main objective of this systematic review was to examine the evidence for the association between genetic polymorphisms and food allergy and identify areas that need further investigation.

2 | METHODS

This systematic review was conducted according to a previously developed protocol registered on the international prospective register of systematic reviews (PROSPERO) and reported according to the PRISMA checklist.[10]

2.1 | Search methods for identification of studies

We searched three databases: MEDLINE (Ovid), EMBASE (Ovid) and PubMed for references using MeSH terms and thesaurus/keywords on 9 January 2018. PubMed was searched only using keywords to retrieve electronic publications and papers not yet indexed in MEDLINE or EMBASE. Results were limited to English language and to studies of children aged 0-18 years old. The search strategy was formulated with the help of an experienced librarian at the Royal Children's Hospital and was first developed in MEDLINE (Ovid) and adapted in other databases. The complete search terms and strategies used are listed in the Online Repository Tables S1-S2.

We additionally hand-searched reference lists of reviews and meta-analyses to include any citations that contained information on genetic association of food allergy not captured by the above strategy.

2.2 | Inclusion criteria of studies

2.2.1 | Type of studies

We included cross-sectional studies, case-control studies, prospective, retrospective longitudinal studies (cohorts and case-control studies), family linkage studies, sibling-pair studies and randomized control trials in our search strategy.

However, only studies that fulfilled the following criteria were included in our final review:

- The presence of unaffected nonatopic control groups in study design.
- Study was carried out in children. Studies that spanned childhood and adulthood were also included.
- Studies examined association between food allergy and single nucleotide polymorphisms (SNPs), haplotypes or copy number variations (CNVs).

Case reports and case series were excluded. These often described rare mutations among individual patients with food allergy. Systematic reviews, meta-analyses, conference abstracts, nonoriginal articles (comments, editorials and book chapters) and animal studies were also excluded. Studies carried out in patients with other pre-existing diseases (such as those with food protein-induced enterocolitis syndrome, autism, eosinophilic esophagitis or any other conditions) apart from food allergy were also excluded.

2.2.2 | Type of outcomes

The main outcome of the systematic review is clinical food allergy. Studies were included if food allergy diagnosis was determined by an (a) oral food challenge or (b) a combination of positive skin prick test and/or specific IgE levels and information on history of food allergy.

2.3 | Quality assessment

Study quality was assessed by a points scoring system comprising of study reproducibility, study design and statistical analyses, adapted from previous studies.[11,12] These studies based their quality assessments on published checklist and recommendations on replicating genotype-phenotype associations[13] and design of genetic studies in complex diseases.[14] Risk of bias was assessed as a measure of study quality but was not used as a basis for inclusion or exclusion of studies. Full details on the criteria for quality assessment and scoring system are included in the Online Repository Table S3.

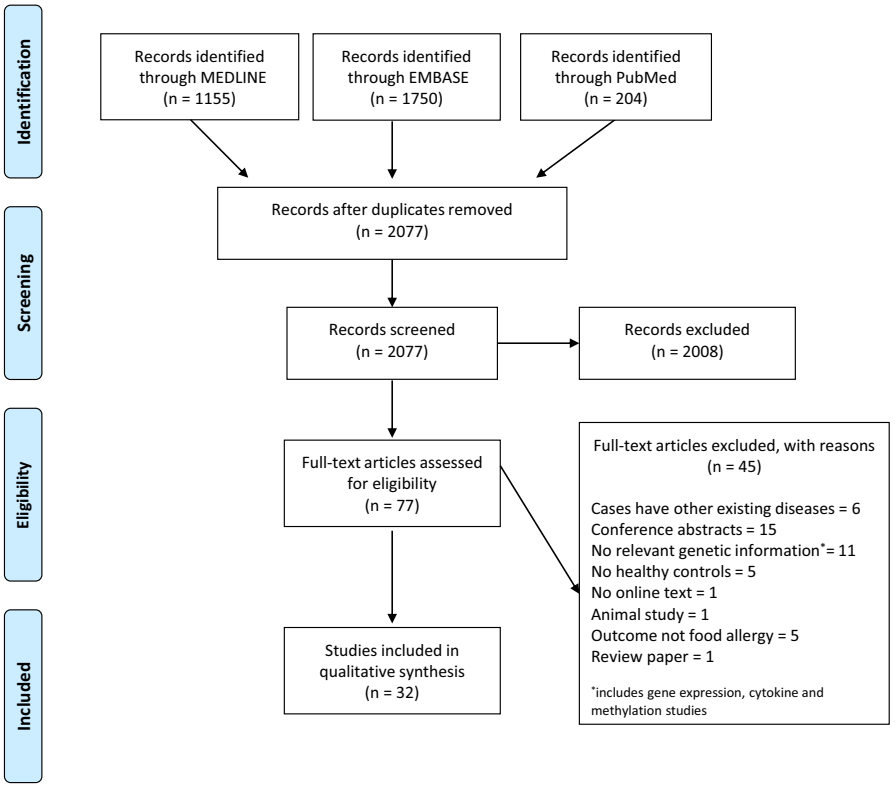

FIGURE 1 Flow chart of literature search process according to PRISMA 2009 flow diagram

2.4 | Data collection and synthesis

Two reviewers (NS and YW) independently screened the title and abstracts of all retrieved citations against the predetermined inclusion and exclusion criteria. Where there was a discrepancy in labelling of included studies, the full text was reviewed by the same reviewers. Eligible papers were scrutinized to extract relevant data and assessed for study quality by two reviewers (NS and VS).

Data were extracted from each paper and compiled for each gene. We reported odds ratios with 95% confidence intervals and, where available, *P*-values for association with food allergy as reported by the original paper.

We chose to report our findings in a narrative manner as there were insufficient data to carry out pooled meta-analyses. In studies where several outcomes (eg asthma, eczema) were studied in addition to food allergy or its subtypes, only the results relevant to food allergy and/or its subtypes were included in the final summary of reported associations. In studies where both atopic controls and nonatopic controls were used, only data pertaining to unaffected nonatopic controls were shown.

3 | RESULTS

3.1 | Characteristics of included studies

A total of 32 articles out of 2088 reviewed met our eligibility criteria (Figure 1). The characteristics of included studies are summarized in Online Repository Table S4.

Two of these studies were gene-environment interaction studies, and these studies reported that the genetic associations were only relevant in the presence of mentioned particular environmental component.[15,16]

We also identified five GWAS with either food or peanut allergy as an outcome. Four of these were carried out in children,[17-20] and the other was carried out in a population across the ages of 1-93 years.[21]

The remaining 25 articles were candidate gene studies, with 14 studies examining food allergy generally, whereas three looked at cow's milk allergy and eight at peanut allergy specifically. Of the 25 candidate gene studies, three of these studies were carried out in a population across ages ranging from 1 to 61 years old, while the remaining 22 studies were in children and young adults under 21 years of age.

Included studies were of varying sample sizes with the smallest study having 30 food allergy Caucasian cases and 35 nonallergic Caucasian controls,[22] while the largest study was a GWAS with 2197 European subjects (671 with food allergy, 144 nonallergic nonsensitized controls and 1382 European controls of uncertain phenotype).[17] The majority (n = 11) of included studies were conducted in only "Caucasian," "European" or "White" populations,[19,21-30] whereas 11 studies were carried out in predominantly Caucasian populations alongside other ethnicities ("Asians," "Mixed" and "African American").[15-18,20,31-36] Four others were carried out in Japanese populations,[37-40] one was carried out in a Taiwanese population,[41] and there were five studies where ethnicity was not mentioned.[42-46]

3.2 | Quality assessment

A detailed assessment of study quality can be found in the Online Repository Table S3. Seventeen of the included studies were of low

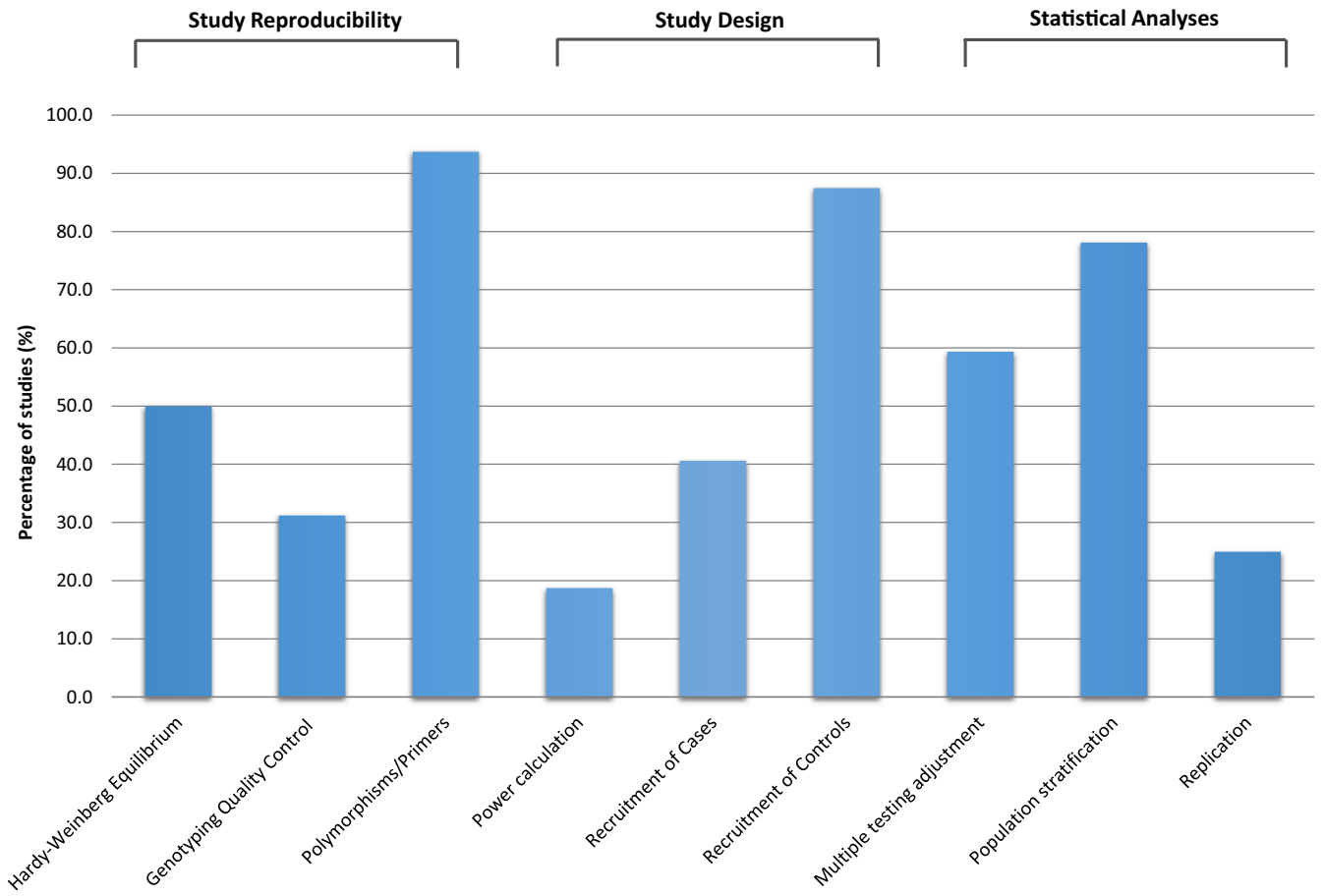

FIGURE 2 Percentage of studies that meet each of the criteria in risk of bias assessment

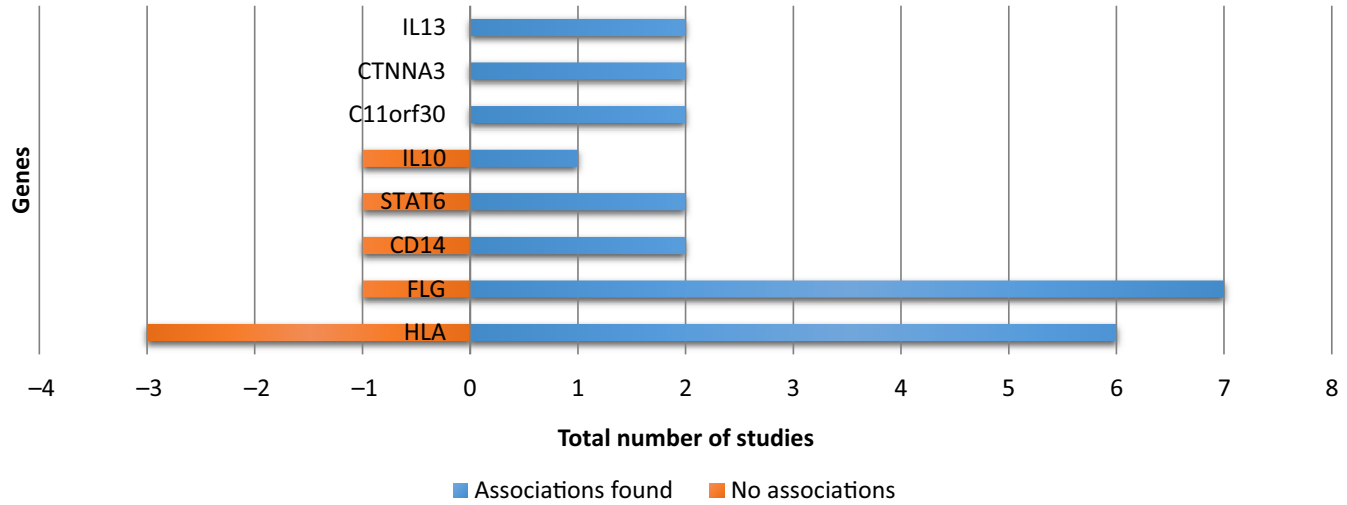

FIGURE 3 Genes/gene regions that were investigated in more than one study and their associations with any type of food allergy. Studies were classified as showing an association if they are associated with any food allergy after multiple testing correction. This included studies that showed suggestive or marginal significant associations. *P* values used were those determined by each study

quality, 10 were of moderate quality, and the remaining five scored highly. Only half of the studies provided information on Hardy-Weinberg equilibrium (HWE) assessment (Figure 2). In terms of study design, few studies (n = 6) included a measure of statistical power as part of their study. 78% of the studies included assessment of population stratification, including those that were not scored on these criteria as they restricted their analyses to one population group (Figure 2). Several studies carried out meta-analyses with additional

TABLE 1 Summary of investigated genes in included studies

Genes of interest	Author	SNPs/CNVs/alleles	Study type	Outcome	OR (95% CI)	*P*-value[a]	Within-study replication[a,b,c]	Cross-study replication[d,e,f]
ABCB11	Hong, 2015[17]	rs16823014	GWAS	Egg allergy	No ORs given	4.4×10^{-6}	N	
ARHGAP24	Asai, 2017[21]	rs744597	GWAS - Meta-analyses	Peanut allergy	0.61 (0.5-0.74)	3.98×10^{-7}		
ATP10A	Martino, 2017[20]	rs17555239	GWAS	Peanut allergy	2.58	3×10^{-5}	OR = 0.79, *P* = 0.131	
BCAS1	Martino, 2017[20]	rs11700330	GWAS	Peanut allergy	0.23	3×10^{-6}	N	
C11orf30/LRRC32	Marenholz, 2017[19]	rs2212434	GWAS	Food allergy	1.29	3.4×10^{-4}	OR = 1.47, $P = 8.2 \times 10^{-5}$ (Replication 1) *P* = 1.4 × 10−4 (Replication 2)	N
C11orf30/LRRC32	Hirota, 2017[38]	rs11236809	Candidate gene	Food allergy	1.34 (1.14-1.59)	0.00056	OR = 1.33 (1.08-1.63), *P* = 0.0096, $P_{combined}$ = 0.000014	N
C11orf30/ LOC101928813	Asai, 2017[21]	rs7936434	GWAS - Meta-analyses	Peanut allergy	1.58 (1.32-1.9)	5.17×10^{-7}		
CCDC80	Hirota, 2017[38]	rs12634229	Candidate gene	Food allergy	1.26 (1.08-1.46)	0.0039	OR = 1.24 (1.02-1.52), *P* = 0.030, $P_{combined}$ = 0.00028	
CD14	Campos, 2007[37]	-159 (rs2569190)	Candidate gene	Food allergy	No ORs given, only frequencies	0.8		✓ (33, 36)
CD14	Campos, 2007[37]	-550 (rs5744455)	Candidate gene	Food allergy	No ORs given, only frequencies	0.8		N
CD14	Dreskin, 2011[33]	rs2569193	Candidate gene	Peanut allergy	1.33 (0.53-3.34)	0.54		N
CD14	Dreskin, 2011[33]	rs2569190	Candidate gene	Peanut allergy	1.97 (1.02-3.79)	0.04		✓ (36) ✗ (37)
CD14	Woo, 2003[36]	−159 C/T (rs2569190)	Candidate gene	Food allergy	1.7 (1.1-2.8)	0.03		✓ (33) ✗ (37)
CHCHD3/EXOC4	Asai, 2017[21]	rs78048444	GWAS	Peanut allergy	0.22 (0.12-0.39)	5.44×10^{-7}		
CLEC16A/DEXI	Hirota, 2017[38]	rs2041733	Candidate gene	Food allergy	1.15 (0.99-1.35)	0.074	OR = 1.18 (0.96-1.45), *P* = 0.12, $P_{combined}$ = 0.019	
COG7	Hong, 2015[17]	rs250585	GWAS	Egg allergy	No ORs given	3.8×10^{-6}	N	
CTNNA3	Asai, 2017[21]	rs7475217	GWAS	Peanut allergy	1.64 (1.35-1.98)	3.58×10^{-7}		N
CTNNA3	Li, 2015[18]	chr10:68282970-68284017 chr10:68383827-68407077	GWAS	Food allergy		0.0184	*P* = 0.0206	N
EMCN	Hong, 2015[17]	rs1318710	GWAS	Food allergy	No ORs given	2.6×10^{-6}	N	
FAM117A	Hong, 2015[17]	rs9898058	GWAS	Cow's milk allergy	No ORs given	1.1×10^{-6}	N	

(Continues)

TABLE 1 (Continued)

Genes of interest	Author	SNPs/CNVs/alleles	Study type	Outcome	OR (95% CI)	*P*-value[a]	Within-study replication[a,b,c]	Cross-study replication[d,e,f]
FcyRIIa	Pawlik, 2004[29]	Not given	Candidate gene	Food allergy	No ORs given, only frequencies	None significant (*P*-values not given)		
FLG	Brown, 2011[25]	Combined null genotype R501X and 2282del4	Candidate gene	Peanut allergy	English: 3.2 (1.4-7.2) English, Dutch, Irish: 5.3 (2.8-10.2)	0.0251 3.0×10^{-6}	N	N
FLG	Brown, 2011[25]	Combined null genotype R501X, 2282del4, R2447X and S3247X	Candidate gene	Peanut allergy	Dutch: 3.5 (1.1-11.4) Irish: 3.3 (1.0-11.7)	0.0335 0.0640	OR = 1.9 (1.4-2.6), $P = 5.4 \times 10^{-5}$	N
FLG	Hirota, 2017[38]	rs6696556	Candidate gene	Food allergy	1.05 (0.84-1.31)	0.68	OR = 1.15 (0.86-1.54), $P = 0.37$, $P_{combined} = 0.39$	N
FLG	Hirota, 2017[38]	p.S2889*	Candidate gene	Food allergy	2.32 (1.37-3.98)	0.001	Replication: OR = 2.41 (1.27-4.49), $P = 0.0049$ Combined: OR = 2.36 (1.58-3.52), $P = 0.000015$	N
FLG	Hirota, 2017[38]	6 FLG null variants, c.3321delA, p.Q1701*, p.S2554*, p.S2889*, p.S3296* and p.K4022*	Candidate gene	Food allergy	1.42 (1.04-1.92)	0.024	Replication: OR = 2.04 (1.38-3.01), $P = 0.00035$ Combined: OR = 1.63 (1.28-2.07), $P = 0.000055$	N
FLG	Savilahti, 2010[45]	5 filaggrin null mutations (del22824, 501-C/T, R2447X, S3247 and 3702delG)	Candidate gene	Cow's milk allergy	No ORs given, only frequencies	None significant ($P > 0.003$)		✓ (30, 46)
FLG	Venkataraman, 2014[46]	5 polymorphisms (R501X, 2282del4, S3247X, 3702delG and R2447X)	Candidate gene	Food allergy	10 y: 2.9 (1.2-7.0) 18 y: 2.5 (1.2- 5.3)	0.022 0.032		✓ (30) ✗ (45)
FLG	Brough, 2014[15]	Combined null mutations R501X, S3247X, R2447X, 2282del4, 3673delC and 3702delG	GxE	Peanut allergy	Univariate: 2.70 (0.9-8.0) Multivariate: 3.2 (1.1-9.8)	0.07 0.04		N
FLG	Tan, 2012[30]	R501X, 2282del4, R2447X, S3247X and 3702delG	Candidate gene	Food allergy	3.2 (1.2-8.5)	0.016 (0.055 after adjusting for eczema)		✓ (46) ✗ (45)
FLG	Asai, 2013[24]	Combined rs61816761, rs41370446, rs138726443 and rs150597413	Candidate gene	Peanut allergy	1.96 (1.49-2.58)	5.12×10^{-7}		N

(Continues)

TABLE 1 (Continued)

Genes of interest	Author	SNPs/CNVs/alleles	Study type	Outcome	OR (95% CI)	*P*-value[a]	Within-study replication[a,b,c]	Cross-study replication[d,e,f]
FLG-AS1	Marenholz, 2017[19]	rs12123821	GWAS	Food allergy	2.55	8.4×10^{-10}	OR = 2.86, $P = 6.1 \times 10^{-7}$ (Replication 1)	N
FXR1	Martino, 2017[20]	rs6763069	GWAS	Peanut allergy	0.38	2×10^{-5}	N	
GC	Koplin, 2016[16]	Combined rs7041 and rs4588	GxE	Food Allergy	6.0 (0.9-38.9)	$P_{interaction} = 0.014$		
GLB1	Hirota, 2017[38]	rs6780220	Candidate gene	Food allergy	1.40 (1.21-1.62)	0.0000082	OR = 1.20 (0.99-1.45), $P = 0.064$ $P_{combined} = 0.0000025$	
HLA	Martino, 2017[20]	Amino acid polymorphisms at position 37	GWAS	Peanut allergy	0.3 (0.16-0.55)	9.8×10^{-5}	N	N
HLA	Martino, 2017[20]	Amino acid polymorphisms at position 71	GWAS	Peanut allergy	0.34 (0.19-0.59)	1.5×10^{-4}	N	✓ (17)
HLA	Savilahti, 2010[45]	HLA class II haplotypes (DQB1, DRB1 and DQA1)	Candidate gene	Cow's milk allergy	No ORs given, only frequencies	None significant ($P > 0.003$)		N
HLA	Howell, 1998[27]	DRB1*08	Candidate gene	Peanut allergy	No ORs given, only frequencies	0.0021 ($P_{corrected} = 0.027$)		N
HLA	Howell, 1998[27]	DBR1*08/12 (tyr16)	Candidate gene	Peanut allergy	No ORs given, only frequencies	0.0023 ($P_{corrected} = 0.029$)		N
HLA	Howell, 1998[27]	DQB1*04	Candidate gene	Peanut allergy	No ORs given, only frequencies	0.00042 ($P_{corrected} = 0.0029$)		N
HLA	Shreffler, 2006[35]	DR11	Candidate gene	Peanut allergy	No ORs given, only frequencies	0.07 ($P_{corrected} = 1.3$)		N
HLA	Shreffler, 2006[35]	DQ7	Candidate gene	Peanut allergy	No ORs given, only frequencies	0.04 ($P_{corrected} = 0.3$)		N
HLA	Shreffler, 2006[35]	6 DQ serotypes (DQ2, DQ4, DQ5, DQ6, DQ8 and DQ9) and 17 DR allele groups (DR1, DR4, DR7, DR8, DR9, DR10, DR12, DR13, DR14, DR15, DR16, DR17, DR18, DR51, DR52, DR53 and DR103)	Candidate gene	Peanut allergy	No ORs given, only frequencies	None significant ($P/P_{corrected} > 0.05$)		N
HLA- DQB1	Madore, 2013[28]	DQB1*06:03P	Candidate gene	Peanut allergy	2.59 (1.56-4.44)	1.6×10^{-4}, $P_c = 1.9 \times 10^{-3}$		N
HLA- DQB1	Madore, 2013[28]	DQB1*02	Candidate gene	Peanut allergy	0.12 (0.07-0.21)	1.1×10^{-16}, $P_{corrected} = 1.3 \times 10^{-15}$		N
HLA- DQB1	Madore, 2013[28]	DQB1*03:02P	Candidate gene	Peanut allergy	0.52 (0.34-0.79)	2.2×10^{-3}, $P_{corrected} = 2.6 \times 10^{-2}$		N
HLA- DQB1	Madore, 2013[28]	DQB1*05	Candidate gene	Peanut allergy	0.21 (0.08-0.50)	2.5×10^{-4}, $P_{corrected} = 3.0 \times 10^{-3}$		N

(Continues)

TABLE 1 (Continued)

Genes of interest	Author	SNPs/CNVs/alleles	Study type	Outcome	OR (95% CI)	*P*-value[a]	Within-study replication[a,b,c]	Cross-study replication[d,e,f]
HLA- DQB1	Madore, 2013[28]	DQB1*05:01P	Candidate gene	Peanut allergy	0.25 (0.13-0.47)	7.7×10^{-6}, $P_{corrected} = 9.3 \times 10^{-5}$		N
HLA-A, B, DRB1, DQB1	Hand, 2004[34]	B*07, DRB1*11	Candidate gene	Nut allergy	No ORs given, only frequencies	None significant ($P > 0.05$)		N
HLA-A, B, DRB1, DQB1	Hand, 2004[34]	DRB1*13	Candidate gene	Nut allergy	No ORs given, only frequencies	<0.05 ($P_{corrected} = 0.82$)		N
HLA-A, B, DRB1, DQB1	Hand, 2004[34]	DQB1*06	Candidate gene	Nut allergy	No ORs given, only frequencies	<0.01 ($P_{corrected} = 0.37$)		N
HLA-B	Li, 2015[18]	chr6:31300691-31304663	GWAS	Food allergy	Not given	Not given	$P = 0.026$, $P_{combined} = 0.063$	N
HLA-DQB1	Marenholz, 2017[19]	rs9273440	GWAS	Peanut allergy	0.66	6.6×10^{-7}	OR = 0.45, $P = 3.8 \times 10^{-6}$ (Replication 1)	N
HLA-DQB1 and HLA-DQA2	Hong, 2015[17]	rs9275596	GWAS	Peanut allergy	European: 1.7 (1.4-2.1) Non-European: 1.2 (0.8-1.8)	6.8×10^{-10} 0.327	OR = 1.7 (1.1-2.6), $P = 0.022$ OR = 0.6 (0.2-1.3), $P = 0.176$	N
HLA-DRA	Hong, 2015[17]	rs7192	GWAS	Peanut allergy	European: 1.7 (1.4-2.1) Non-European: 1.2 (0.8-1.8)	5.5×10^{-8} 0.198	OR = 1.8 (1.2-2.7), $P = 0.005$ OR = 1.4 (0.7-3.1), $P = 0.375$	N
HMGA2|LLPH	Hong, 2015[17]	rs10878354	GWAS	Peanut allergy	Not given	5.1×10^{-6}	N	
IDO1 and IDO2	Buyuktiryaki, 2016[43]	10 SNPs: rs3808606, rs3824259, rs10089084, rs6991530, rs10504013 rs11992749, rs10109853, rs4503083, rs2955903 and rs7820268	Candidate gene	Food allergy	No ORs given, only frequencies	None significant (*P* values >0.05)		
IER5L	Martino, 2017[20]	rs4240433	GWAS	Peanut allergy	3.61	7×10^{-6}	OR = 0.83, 0.316	
IL10	Abe Jacob, 2013[42]	-1082	Candidate gene	Cow's milk allergy	No ORs given, only frequencies	0.027 ($P_{corrected} = 0.054$)		✗ (41)
IL10	Chen, 2012[41]	-1082 A/G (rs1800896) and -592 A/C (rs1800872)	Candidate gene	Food allergy	No ORs given, only frequencies	0.994 0.770		✓ (42)
IL13	Ashley, 2017[32]	rs1295686	Candidate gene	Food allergy	1.75 (1.20-2.53)	0.003	OR = 1.37 (1.03-1.82), $P = 0.03$	
KIF3A/IL13	Hirota, 2017[38]	rs1295686	Candidate gene	Food allergy	1.44 (1.23-1.68)	0.0000031	OR = 1.34 (1.10-1.64), $P = 0.0038$, $P_{combined} = 0.000000067$	

(Continues)

TABLE 1 (Continued)

Genes of interest	Author	SNPs/CNVs/alleles	Study type	Outcome	OR (95% CI)	*P*-value[a]	Within-study replication[a,b,c]	Cross-study replication[d,e,f]
IL2/IL21	Hirota, 2017[38]	rs17389644	Candidate gene	Food allergy	1.14 (0.90-1.44)	0.28	OR = 1.49 (1.13-1.97), $P = 0.0049$, $P_{combined} = 0.0096$	
IL26	Martino, 2017[20]	rs7300806	GWAS	Peanut allergy	0.28	1×10^{-5}	OR = 0.82, $P = 0.319$	
IL28B	Gaudieri, 2012[22]	rs12979860	Candidate gene	Food allergy	Cohort 1: 4.56 (1.7-12.6) Cohort 2: 3.0 (1.8-5.2)	0.004 0.04		
IL4/KIF3A	Marenholz, 2017[19]	rs11949166	GWAS	Food allergy	0.6	1.2×10^{-13}	OR = 0.69, $P = 3.0 \times 10^{-5}$ (Replication 1)	
IMPAD1|LOC286177	Hong, 2015[17]	rs7833294	GWAS	Cow's milk allergy	No ORs given	7.3×10^{-6}	N	
ITIH5L	Hong, 2015[17]	rs5961136	GWAS	Egg allergy	No ORs given	2.4×10^{-6}	N	
LINGO2	Martino, 2017[20]	rs10812871	GWAS	Peanut allergy	0.38	4×10^{-5}	OR = 0.68 $P = 0.014$*	
LMX1A	Martino, 2017[20]	rs6686894	GWAS	Peanut allergy	0.06	4×10^{-7}	OR = 1.29, $P = 0.280$	
LOC100129104|ZFAT	Hong, 2015[17]	rs4584173	GWAS	Peanut allergy	No ORs given	3.6×10^{-6}	N	
LOC100289292|ETAA1	Hong, 2015[17]	rs17032597	GWAS	Cow's milk allergy	No ORs given	1.6×10^{-6}	N	
LOC100289677|TP53TG1	Hong, 2015[17]	rs6942407	GWAS	Food allergy	No ORs given	8.2×10^{-6}	N	
LOC645314|SLC39A10	Hong, 2015[17]	rs777717	GWAS	Food allergy	No ORs given	4.7×10^{-6}	N	
LOC729993|ERCC4	Hong, 2015[17]	rs6498482	GWAS	Egg allergy	No ORs given	4.8×10^{-6}	N	
LSP1	Hong, 2015[17]	rs78405116	GWAS	Cow's milk allergy	No ORs given	1.7×10^{-6}	N	
LUZP2	Li, 2015[18]	chr11:2477896124783183 chr11:24412621-24551109	GWAS	Food allergy	No ORs given, only frequencies	0.0226	$P = 0.0153$	
MACROD2	Li, 2015[18]	chr20:1510419315126507 chr20:14713890-14727386	GWAS	Food allergy	No ORs given, only frequencies	3.37×10^{-3}	$P = 1.41 \times 10^{-3}$	
MDN1	Martino, 2017[20]	rs9362681	GWAS	Peanut allergy	2.83	1×10^{-5}	OR = 1.43, $P = 0.037$	
NAT2	Gawronska-Szklarz, 2001[26]	NAT2*4 (fast acetylator), NAT2*5, NAT2*6 and NAT2*7 (slow acetylators)	Candidate gene	Food allergy	No ORs given, only frequencies	$P < 0.001$		
NAV2	Martino, 2017[20]	rs2439871	GWAS	Peanut allergy	0.38	1×10^{-5}	OR = 0.94, $P = 0.723$	
NLRP3	Hitomi, 2009[39]	rs12079994	Candidate gene	Food-induced anaphylaxis	1.81 (1.09-2.99)	0.021		
NLRP3	Hitomi, 2009[39]	rs4925650	Candidate gene	Food-induced anaphylaxis	1.77 (1.26-2.49)	0.00091		
NLRP3	Hitomi, 2009[39]	rs3806265	Candidate gene	Food-induced anaphylaxis	1.71 (1.20-2.43)	0.0029		
NLRP3	Hitomi, 2009[39]	rs4612666	Candidate gene	Food-induced anaphylaxis	1.81 (1.27-2.56)	0.00086		

(Continues)

TABLE 1 (Continued)

Genes of interest	Author	SNPs/CNVs/alleles	Study type	Outcome	OR (95% CI)	*P*-value[a]	Within-study replication[a,b,c]	Cross-study replication[d,e,f]
NLRP3	Hitomi, 2009[39]	rs10925026	Candidate gene	Food-induced anaphylaxis	1.53 (1.09-2.16)	0.013		
NLRP3	Hitomi, 2009[39]	rs10754558	Candidate gene	Food-induced anaphylaxis	1.80 (1.28-2.54)	0.00068		
NLRP3	Hitomi, 2009[39]	rs10733112	Candidate gene	Food-induced anaphylaxis	1.71 (1.21-2.40)	0.0021		
NLRP3	Hitomi, 2009[39]	rs2027432, rs4925648, rs12048215, rs10754555, rs10925019, rs4925654, rs12565738 and rs4378247	Candidate gene	Food allergy	No ORs given	None significant ($P > 0.05$)		
ODZ3	Li, 2015[18]	chr4:183271349183291465 chr4:183559306-183565618	GWAS	Food allergy	No ORs given, only frequencies	0.0116	$P = 0.018$	
OR10A3/NLRP10	Hirota, 2017[38]	rs878860	Candidate gene	Food allergy	1.10 (0.95-1.27)	0.21	OR = 1.29 (1.07-1.57), $P = 0.01$, $P_{combined} = 0.01$	
OVOL1	Hirota, 2017[38]	rs593982	Candidate gene	Food allergy	1.23 (1.06-1.42)	0.0049	OR = 1.04 (0.86-1.26), $P = 0.72$, $P_{combined} = 0.016$	
PAFAH1B1	Martino, 2017[20]	rs8077351	GWAS	Peanut allergy	0.05	3×10^{-5}	OR = 1.07, $P = 0.820$	
PAX2	Martino, 2017[20]	rs6584390	GWAS	Peanut allergy	3.56	4×10^{-5}	OR = 1.03, $P = 0.864$	
PLAGL1	Martino, 2017[20]	rs6928827	GWAS	Peanut allergy	13.98	1×10^{-7}	OR = 0.77, $P = 0.292$	
PTPN22	Savilahti, 2010[45]	R620W (rs2476601)	Candidate gene	Cow's milk allergy	No ORs given, only frequencies	None significant (*P*-value >0.003)		
PYROXD1	Martino, 2017[20]	rs7131777	GWAS	Peanut allergy	2.55	4×10^{-5}	N	
RBFOX1	Li, 2015[18]	chr16:71266297196046 chr16:6763216-6801846	GWAS	Food allergy	No ORs given, only frequencies	4.72×10^{-3}	$P = 0.9989$	
RGS21	Martino, 2017[20]	rs12142904	GWAS	Peanut allergy	3.51	5×10^{-6}	OR = 1.02, $P = 0.905$	
RHOBTB1	Hong, 2015[17]	rs10994607	GWAS	Food allergy	No ORs given	7.1×10^{-6}	N	
RHOBTB1|TMEM26	Hong, 2015[17]	rs10994613	GWAS	Cow's milk allergy	No ORs given	4.8×10^{-6}	N	
RIMS2	Martino, 2017[20]	rs16870788	GWAS	Peanut allergy	3.58	3×10^{-5}	OR = 0.93, $P = 0.734$	
RNF130	Martino, 2017[20]	rs864481	GWAS	Peanut allergy	2.91	5×10^{-5}	OR = 1.09, $P = 0.681$	
SALL3	Martino, 2017[20]	rs73971133	GWAS	Peanut allergy	0.07	3×10^{-5}	OR = 0.87, $P = 0.723$	
SERPINB7	Marenholz, 2017[19]	rs12964116	GWAS	Food allergy	1.9	5.7×10^{-6}	OR = 1.69, $P = 9.4 \times 10^{-3}$ (Replication 1) $P = 0.010$ (Replication 2)	

(Continues)

TABLE 1 (Continued)

Genes of interest	Author	SNPs/CNVs/alleles	Study type	Outcome	OR (95% CI)	*P*-value[a]	Within-study replication[a,b,c]	Cross-study replication[d,e,f]
SERPINB7/B2	Marenholz, 2017[19]	rs1243064	GWAS	Egg allergy	1.65	1.6×10^{-7}	OR = 1.21, *P* = 0.028 (Replication 1) *P* = 0.15 (Replication 2)	
SGCD	Hong, 2015[17]	rs7717393	GWAS	Egg allergy	No ORs given	1.4×10^{-6}	N	
SKAP1	Asai, 2017[18]	rs16955960	GWAS	Peanut allergy	2.06 (1.54-2.75)	1.01×10^{-6}		
SLC2A9	Martino, 2017[20]	rs10018666	GWAS	Peanut allergy	5.9	4×10^{-8}	OR = 1.18, *P* = 0.360	
SORBS2	Martino, 2017[20]	rs57144668	GWAS	Peanut allergy	0.37	3×10^{-5}	OR = 1.50, *P* = 0.014	
SPINK5	Ashley, 2017[31]	77 tag-SNPs within a region of ~263 kb capturing 387 alleles with LD of $r^2 \geq 0.8$	Candidate gene	Food allergy	2.95 (1.49-5.83)	0.001	OR = 1.58 (1.13-2.20), *P* = 0.007	
SSBP3|ACOT11	Hong, 2015[17]	rs12121623	GWAS	Food allergy	No ORs given	3.1×10^{-7}	N	
STAT6	Tamura, 2003[40]	G2964A (rs324015)	Candidate gene	Food-related anaphylaxis	No ORs given, only frequencies	0.4974		✓ (23)
STAT6	Amoli, 2002[23]	2964 G/A 3′UTR (rs324015)	Candidate gene	Nut allergy	2.9 (1.7- 4.9)	< 0.0001		✗ (40)
STAT6	Hirota, 2017[38]	rs167769	Candidate gene	Food allergy	1.26 (1.06-1.50)	0.0082	OR = 1.24 (0.99-1.56), *P* = 0.06, $P_{combined} = 0.0014$	N
STXBP6|NOVA1	Hong, 2015[17]	rs862942	GWAS	Peanut allergy	No ORs given	3.0×10^{-6}	N	
SV2C	Martino, 2017[20]	rs10474468	GWAS	Peanut allergy	0.37	5×10^{-5}	OR = 0.84, *P* = 0.261	
TES	Martino, 2017[20]	rs73220497	GWAS	Peanut allergy	0.06	3×10^{-5}	OR = 1.04, *P* = 0.891	
TGFb1	Abe Jacob, 2013[42]	-509C/T	Candidate gene	Cow's milk allergy	No ORs given, only frequencies	0.6419		
TLR2	Galli, 2010[44]	R753Q (rs5743708)	Candidate gene	Cow's milk allergy	No ORs given, only frequencies	None significant (*P* values >0.05)		
TLR4	Galli, 2010[44]	D299G (rs4986790)	Candidate gene	Cow's milk allergy	No ORs given, only frequencies	None significant (*P* values >0.05)		
TMEM232/SLC25A46	Hirota, 2017[38]	rs9326801	Candidate gene	Food allergy	1.33 (1.09-1.61)	0.0037	OR = 0.98 (0.75-1.27), *P* = 0.87, $P_{combined} = 0.031$	
TNFRSF6B/ZGPAT	Hirota, 2017[38]	rs6010620	Candidate gene	Food allergy	1.11 (0.95-1.29)	0.19	OR = 1.19 (0.98-1.46), *P* = 0.082, $P_{combined} = 0.039$	
TSLP/WDR36	Hirota, 2017[38]	rs3806932	Candidate gene	Food allergy	1.19 (1.02-1.40)	0.032	OR = 1.15 (0.94-1.42), *P* = 0.19, $P_{combined} = 0.012$	

(Continues)

TABLE 1 (Continued)

Genes of interest	Author	SNPs/CNVs/alleles	Study type	Outcome	OR (95% CI)	*P*-value[a]	Within-study replication[a,b,c]	Cross-study replication[d,e,f]
ZNF365	Hirota, 2017[38]	rs10995251	Candidate gene	Food allergy	1.32 (1.14-1.53)	0.00017	OR = 1.15 (0.95-1.39), $P = 0.18$, $P_{combined} = 0.00013$	
ZNF652	Hirota, 2017[38]	rs16948048	Candidate gene	Food allergy	1.20 (0.97-1.47)	0.093	OR = 1.41 (1.08-1.82), $P = 0.0096$ $P_{combined} = 0.0039$	

CNVs, copy number variations; GWAS, genome-wide association study; GxE, gene-environment interaction studies; OR, odds ratios; SNPs, single nucleotide polymorphisms.
[a] $P_{combined}$ refers to *P*-values obtained from the combination of discovery and replication cohort, as given in the respective studies. $P_{corrected}$ refers to *P*-values after multiple testing correction.
[b] Empty rows indicate study did not include a replication cohort.
[c] N denotes studies have replication cohort but SNP/allele was not investigated in replication cohort.
[d] Empty rows indicate no other studies investigated same gene.
[e] N denotes studies that investigated the same gene, but the investigated SNP/allele differ among studies.
[f] ✓ Indicates findings are associated with food allergy in cited study. ✗ Indicates findings are not associated with food allergy in cited study.

cohorts instead of having a replication cohort, but the results were not included in this review. Only 25% of the studies carried out an independent replication cohort to validate their findings.

3.3 | Genes investigated in included studies

We identified seven gene regions investigated in more than one study and presented the congruency of their findings here. A summary of these gene regions and the evidence of association with food allergy is shown in Figure 3. A detailed compilation of genes and SNPs from all eligible studies is provided in Table 1.

3.3.1 | HLA

Human leukocyte antigen (HLA) complex has been one of the most commonly studied gene regions in current food allergy research. This gene has been investigated by nine studies, although with inconsistent findings. Studies investigating *HLA* were widely heterogeneous primarily due to the highly polymorphic nature of the HLA region and the different variant classes. Some studies analysed the classical two- or four-digit alleles, while others analysed the specific HLA protein, amino acid polymorphisms or SNPs within the gene. A study by Li et al,[18] however, was the only study that investigated candidate genes as well as CNV and CNV region (CNVR) in a genome-wide data set. In its candidate gene analysis, rare CNVs of duplication in the gene HLA-B at chr6:31300691-31304663 were detected in two food allergy cases and three control samples.

Associations with SNPs

In the first GWAS of food allergy by Hong et al[17] (n = 2694 post–quality control), no polymorphism in the HLA region was found to reach the genome-wide significance level or suggestive threshold in the discovery cohort with the outcome of "any food allergy" (Table 1). When analysed for specific food allergy such as peanut, egg and milk allergy, two polymorphisms (nonsynonymous mutation rs7192 of *HLA-DRA* and rs9275596 intergenic SNP between *HLA-DQB1* and *HLA-DQA2*) were associated with an increased risk of peanut allergy only, and these findings were replicated in an independent cohort. However, this association was only observed in children of European ancestry and not non-European ancestry. These variants, rs7192 ($r^2 = 0.25$) and rs9275596 ($r^2 = 0.48$), were found to be in linkage disequilibrium with a 3′ UTR variant, rs9273440 of *HLA-DQB1*, which was significantly associated with peanut allergy in another GWAS of food allergy.[19]

Association with broad allele groups

Six of the nine studies that investigated *HLA* found associations with broad allele groups. With the exception of Savilahti et al[45] who focused on cow's milk allergy, the remaining five studies investigated *HLA* in relation to peanut allergy.[20,27,28,34,35] Savilahti et al[45] did not find any significant associations with cow's milk allergy for HLA class II DR and DQ haplotypes. Meanwhile, Howell et al[27] reported an

amino acid variant (DRB1*08/12 - tyr16) and two alleles (DRB1*08, DQB1*04) that showed an increased proportion in peanut allergic individuals compared to controls, even after multiple testing correction. Two other allele groups, DQB1*02 and DQB1*05, were lower in peanut allergy cases compared to controls.[28] Analysis of specific HLA proteins in the same study found a higher frequency of DQB1*06:03P, but a decreased frequency of DQB1*03:02 and DQB1*05:01P in peanut allergy cases compared to controls.[28] The letter "P" added at the end of the allele represents alleles that share the same peptide binding domains.[47]

Apart from the Howell et al and Madore et al studies, three other studies reported an association with peanut allergy, but these associations did not survive multiple testing adjustment[20,34,35] (Table 1). These studies had smaller sample sizes in comparison with other studies which may have contributed to the lack of association. In Shreffler et al's[35] study carried out in discordant sibling pairs, none of the alleles investigated were associated with peanut allergy in 73 cases. However, the DQ7 serotype frequency was higher in sibling controls than those with peanut allergy. In the other study of 84 cases, DRB1*13 and DQB1*06 alleles were higher in cases than controls.[34] The last study found an association between reduced risk of peanut allergy and two amino acid variants, which were in linkage disequilibrium in *HLA-DRB1* (positions 37 and 71).[20] The association between peanut allergy and the variant at position 71 was initially discovered by Hong et al,[17] but it did not remain significant in the replication cohort.

3.3.2 | FLG

Similarly, the gene encoding filaggrin (*FLG*) was also commonly investigated with seven studies investigating association of different *FLG* variants with peanut, cow's milk or food allergy[19,24,25,30,38,45,46] and one study investigating the association in the presence of an environmental exposure (gene-environment interaction).[15] Similar to the studies on *HLA*, these studies tend to investigate different combinations of loss-of-function *FLG* mutations, making direct comparisons between the studies challenging. The combination of mutations investigated for each of these studies is shown in Table 1.

Six studies reported a significant association with either food allergy or peanut allergy in the presence/absence of environmental exposure.[19,24,25,30,38,46] Cases were reported to have a higher proportion of loss-of-function mutations,[24,30] and individuals with loss-of-function mutations[25,30,38,46] or "T" allele of intron variant, rs12123821,[19] were at least two times more likely to have food or peanut allergy than the control group. However, in a birth cohort study where participants were followed up prospectively for 18 years, an association between food allergy and *FLG* mutations was only observed at 10 and 18 years but not at younger ages (at 1, 2 and 4 years old).[46] It may be that *FLG* mutations are less strongly associated with food allergies that predominate in younger children, such as egg and milk.

The study on cow's milk allergy by Savilahti et al[45] did not find any significant associations with cow's milk allergy for any of their investigated *FLG* polymorphisms (combined del22824, 501-C/T, R2447X, S3247 and 3702delG).

One of the identified studies, Brough et al,[15] investigated effect modifications of genetic polymorphisms in *FLG* on the association between peanut allergy and peanut exposure. In this study, 9% of all children (N = 623) had a loss-of-function *FLG* mutations (combined R501X, S3247X, R2447X, 2282del4, 3673delC and/or 3702delG), whereas in peanut allergy cases, 4 out of 20 (20%) carried the loss-of-function *FLG* mutations. In the multivariate model, children with one or more *FLG* mutations had a 3.3 times increased odds of peanut allergy with each natural log (ln [log e]) unit increase in house dust peanut exposure. On the other hand, no association between peanut exposure and peanut allergy or peanut sensitization was observed in children with the wild-type *FLG* genotype.

3.3.3 | CD14

Three small studies (N < 200 subjects in each study) investigated the association of cluster of differentiation 14 (CD14) gene and food allergy.[33,36,37] These studies all investigated the 5′ UTR variant –159 C/T (rs2569190) but obtained conflicting results. Dreskin et al[33] found the C allele to be associated with peanut allergy. Conversely, Woo et al[36] found a higher proportion of T alleles in food allergy cases than the controls in both codominant and dominant-recessive models, while Campos et al[37] found no evidence of an association between this polymorphism and food allergy. However, it is worth noting that the two latter studies were carried out in different populations—the Woo study was carried out in predominantly Caucasian with some mixed ethnicity (African American or others not specified), while the Campos study was carried out in a Japanese population.

3.3.4 | STAT6

Three studies investigated the associations between polymorphisms within gene encoding signal transducer and activator of transcription 6 (*STAT6*) and nut allergy,[23] food allergy[38] or food-related anaphylaxis.[40] The G allele of 3′ UTR variant 2964G/A (rs324015) was found at an increased frequency in Caucasian children with nut allergy.[23] This same variant, however, was not associated with food-related anaphylaxis in Japanese children.[40] The last study on food allergy found an association with a 5′ UTR variant in the STAT6 region, rs167769,[38] which was previously associated with eosinophilic esophagitis.[48]

3.3.5 | IL10

Chen et al[41] and Jacob et al[42] investigated variants at the gene encoding interleukin 10 (*IL10*) in relation to any food and cow's milk allergy, respectively. A common SNP investigated by both studies is the -1082 A/G (rs1800896) variant, a 2 kilo base pair (kb) upstream variant. Jacob et al[42] found that the GG allele for -1082 A/G (rs1800896) was more common in the cow's milk allergy group than

the control group. Moreover, the *IL10* -3575A, *IL10* -2849A, *IL10* -2763C, *IL10* -1082G and *IL10* -592C haplotypes were also higher in cases (10%) than controls (2%). On the other hand, Chen et al[41] did not find an association of any food allergy (milk inclusive) with either the same variant -1082 A/G (rs1800896) or -592 A/C (rs1800872) variant.

3.3.6 | IL13

Two studies found an association between food allergy and interleukin 13 (*IL13*) intron variant, rs1295686.[32,38] Both studies observed an increased risk of food allergy among those with the risk allele (A/T). Interestingly, the studies were carried out in different populations, with the Ashley et al[32] study conducted in a Caucasian population using a tag-SNP selection approach, while the Hirota et al[38] study was done in a Japanese population investigating genes previously associated with atopic dermatitis and/or eosinophilic esophagitis.

3.3.7 | C11orf30/LRRC32

Hirota et al[38] investigated the association of food allergy with 26 genes previously associated with atopic diseases and eosinophilic esophagitis in GWAS. In this study, a locus within the chromosome 11 open reading frame 30/ leucine-rich repeat-containing protein 32 (*C11orf30)/LRRC32*) region was one of 14 loci found to be associated with food allergy at the nominal level ($P < 0.05$). rs11236809, a 500 base pair downstream variant, was associated with food allergy. In another study by Marenholz et al,[19] an intergenic variant (rs2212434) within the same *C11orf30/LRRC32* region was also associated with food allergy in the discovery cohort and two independent replication cohorts. Additionally, Asai et al[21] found an association between peanut allergy and rs7936434, a variant 30kb from *C11orf30*. Collectively, these three studies point towards the association of the region with food or peanut allergy but none investigated the same SNPs for comparison.

3.3.8 | Other genes

There were several other studies that investigated genetic associations with food allergy, namely *NLRP3*,[39] *FcyRIIa*,[29] *IDO*,[43] *NAT2*,[26] *SPINK5*,[31] *IL28B (IFNL3)*,[22] *SERPINB*,[19] *TGFb1*,[42] *TLR2* and *TLR4*.[44]

NLRP3 was not found to be associated with food allergy; however, some of the investigated SNPs were found to be associated with food-related anaphylaxis.[39]

NAT2, *SERPINB* and *SPINK5* were reported to be associated with food allergy in a single study each, while the remaining studies of the other genes found no association. Of particular significance is the *SERPINB* gene cluster, a newly identified region associated with challenge-proven food allergy. The association was identified in a GWAS carried out using data from the German Genetics of Food Allergy Study (GOFA).[19] One of the SNPs located in the intron of *SERPINB*, rs12964116, did not remain significant after multiple testing correction in a GOFA replication cohort, but was associated with food allergy when investigated in a second independent replication cohort. Additionally, *SPINK5* variant rs9325071, which has been shown to decrease expression of *SPINK5* in the skin, was associated with challenge-proven food allergy in both the discovery and replication cohorts.[31]

In Li et al,[18] CNVR in *ODZ3*, *CTNNA3*, *LUZP2*, *RBFOX1* and *MACROD2* was found to be associated with food allergy. The *CTNNA3* region was also associated with peanut allergy in another study, where intron variant, rs7475217, was associated with a reduction in the risk of peanut allergy.[21]

Apart from these genes, the second gene-environment interaction study investigated polymorphisms of the vitamin D-binding protein gene, *GC*, which were found to modify the association between vitamin D levels and food allergy.[16] Vitamin D insufficiency (≤50 nM/L) at 1 year was associated with food allergy in infants with the GG genotype of rs7041, but not in those with GT or TT genotypes. However, the study did not examine for an association between *GC* and food allergy, independently of vitamin D levels.

4 | DISCUSSION

This is the first review to systematically collate genetic association studies of food allergy. Overall, studies were of varied quality and reproducibility of findings for the same SNPs was minimal. This is not particularly surprising given genetic association studies in food allergy are still emerging. While a number of discovery studies did not include a replication phase, it is promising to notice that more recent studies are recognizing the importance of replication in order to minimize publication of false-positive findings. With the exception of two studies published in 2016,[16,43] the remaining eight studies published within the past 3 years all included a replication analysis. Most studies also included an appropriate adjustment for population heterogeneity in the form of a statistical adjustment, an exclusion of mixed/other ethnicities in their statistical analysis and inclusion of ancestry informative markers as genetically inferred ancestry or was mentioned as a limitation of their study. However, several studies failed to address the need for any population adjustment. Assessment of population stratification is essential in genetic studies since any allelic or genotypic frequencies observed may be correlated with ethnicity and not the disease outcome. Apart from population stratification, multiple testing adjustment is also crucial since the absence of multiple corrections may lead to false-positive associations with food allergy. However, 13 of the included studies did not adequately address these criteria.

In this review, we have included studies that have used an OFC as a diagnostic measure for defining food allergy as well as studies using measures of IgE sensitization in conjunction with history of reaction. Out of the 32 included studies, 11 studies defined food allergy based on history of reactions and SPT, 9 used OFC, and the remaining 12 studies used a combination of classifications—an OFC where possible/available and where unavailable; a history of reaction was used instead. Evidently, there is still a paucity of studies

using OFC as a definition for food allergy. Use of SPT and history of reaction alone are likely to increase the chances of misclassification of food allergy cases.

Despite these limitations, reproducible associations with food allergy were found for a limited number of genes. The most reproducible association with food allergy is for the *FLG* loss-of-function mutations, which were independently reported in eight studies. *FLG* encodes for an intermediate filament-associated protein that aggregates keratin intermediate filaments in mammalian epidermis which are important in water retention.[49] A loss-of-function mutation in *FLG* would thus potentially increase skin permeability and enhance allergen penetration through the skin.[50,51] This mechanism has been demonstrated in several mouse model studies.[52-54] *FLG* variants have also been shown to be associated with eczema and other allergic diseases.[55] While there have been several studies investigating *FLG* association with food allergy, we were unable to perform a meta-analysis since only two studies investigated the same set of FLG polymorphisms. Studies of this gene often combine multiple loss-of-function mutations for analysis of association with disease. The combination of loss-of-function mutations investigated differs between studies, often based on the ethnicity of study participants. Nonetheless, currently available data overall support a genuine association between food allergy and *FLG*.

The next most reproducible associations were found between variants at HLA genes, *DQB1* and *DRB1*, and peanut allergy phenotypes. The HLA-DR and HLA-DQ molecules are expressed in several cells with antigen-presenting capability such as B cells, macrophages and monocytes which are known to play a critical role in the development of allergy. One of the key steps to antigen-specific immune responses is antigen presentation by HLA molecules. As these HLA molecules have specific molecular polymorphisms confined to its peptide binding groove, these polymorphisms may alter the binding affinity of antigen-presenting cells for specific peanut peptides.[17] In particular, the polymorphic amino acid residue 71 along with positions 13, 70 and 74 have been shown to affect the binding specificity of pocket 4, therefore influencing the presentation and interaction of peanut antigens.[17,56] Two SNPs in this region which were associated with peanut allergy, rs7192 and rs9275596, were additionally found to affect DNA methylation and thereby expression levels of *HLA-DRB1* and *HLA-DQB1*.[17] The results of this review appear to show a distinction in genetic association based on the type of food. For instance, it is likely that *HLA* plays a causal role in food allergy, with high specificity to peanut allergy.

A recently identified gene,*C11orf30/LRRC32*, has shown promising results for an association with food allergy. The C11orf30/LRRC32 region has previously been associated with eczema,[57-59] asthma,[60,61] serum IgE levels[62] and eosinophilic esophagitis.[48] The *C11orf30* encodes the EMSY protein which is responsible for binding of *BRCA2* cancer susceptibility gene.[63] Given its role in inflammatory diseases, *C11orf30* may play a role in epithelial barrier and differentiation.[57] The neighbouring gene, *LRRC32*, is a surface biomarker expressed on regulatory T cells[64] shown to be important in immune tolerance.[65] One of the investigated SNPs in this region, rs2212434, was associated with food allergy,[19] and an association with eczema was previously identified in a large meta-GWAS on eczema.[66] Another SNP in the region was also found to increase the risk of atopic march (rs2155219, 17 kb away from rs2212434),[67] further supporting the role of this region in allergic diseases.

Collectively, the involvement of several genes in the mechanism of food allergy points towards the complex and multifactorial nature of food allergy. Like other allergic diseases, the genetic architecture of food allergy appears to involve several relatively common genetic variants of low penetrance and variable expressivity, although the role for rare deleterious mutations has not yet been explored. Some of the genes with evidence for association with food allergy have also been shown to be associated with other allergic diseases such as eczema, asthma and allergic rhinitis. Identifying genes uniquely associated with food allergy is therefore challenging. Some genetic variants may increase overall susceptibility to atopy, such as those in *FCER1A*, *STAT6* and *IL13*[68,69] which are associated with total serum IgE. While these variants can manifest as a number of allergic diseases as well as symptomatic and asymptomatic sensitization to foods and aeroallergens, others such as those in *HLA* may be specifically associated with reactions to a particular food such as peanut. As such, it is important for future studies to clarify whether the intention is to focus on genetic risk factors specific to food allergy, including specific food allergies such as peanut allergy, or to investigate shared markers for allergic diseases.

4.1 | Limitations of this systematic review

We restricted our systematic review to paediatric studies since the prevalence of food allergy is known to be the greatest in children compared to adults and the quality of case phenotyping at the population level is higher. We also did not include results of studies that have carried out computer mapping or pathway analyses to find causal food allergy genes. These studies may provide greater insight into other potentially relevant genes that have not been examined in genetic association studies and may be worth pursuing, but is beyond the scope of the review. Several papers[70-72] that were often quoted in narrative reviews as relevant to food allergy genetic associations were excluded from our systematic review. These studies were excluded primarily because they did not include a healthy control group in their study and/or only investigated genetic associations with regard to severity of food allergy and not the absence/presence of food allergy. We were also unable to carry out meta-analysis on the collated data due to the small number of studies of each locus and the fact that studies investigated different polymorphisms at these loci.

5 | CONCLUSIONS

To date, there is relatively strong evidence that food allergy is associated with genetic variants at *FLG*, *HLA* and *IL13*, as well as some

evidence for other variants (*SPINK5*, *SERPINB* and *C11orf30*) that warrant further investigation. Although several studies reported promising data to support associations of genetic variants with food allergy, they were compromised by issues of inadequate sample size, absence of multiple testing correction and population stratification. Future investigations would benefit from having larger numbers to improve power and include replication cohorts to validate findings. Further functional research is also necessary to unravel the mechanisms of action of identified novel gene variants responsible for the observed associations.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

NS, JK and JE were responsible for developing the protocol, search strategy and risk of bias assessment. NS and YW reviewed all titles and abstracts for eligibility against a predetermined set of inclusion criteria. NS reviewed the full text of potentially eligible papers and extracted data from the original papers, including carrying out the quality assessment of included studies. VS checked the accuracy and authenticity of data extracted. DM and KA contributed to the data analysis and interpretation of data. All authors contributed to the drafting and revising of the article for intellectual content and approved the final version of the manuscript.

ORCID

Yichao Wang https://orcid.org/0000-0002-1166-3796
Katrina J. Allen https://orcid.org/0000-0002-1921-4493
Jennifer J. Koplin https://orcid.org/0000-0002-7576-5142

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Online Supplementary Information

Genetic determinants of paediatric food allergy: A systematic review

Noor H. A. Suaini[1,2], Yichao Wang[1,2], Victoria X. Soriano[1,2], David J. Martino[1,2,3], Katrina J. Allen[1,2,4,5], Justine A. Ellis[1,6,7], Jennifer J. Koplin[2,8]

Affiliations:

[1]Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia

[2]Centre for Food and Allergy Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[3]Telethon Kids Institute, University of Western Australia, Perth, Australia.

[4]Department of Allergy and Clinical Immunology, Royal Children's Hospital, Parkville, Victoria, Australia

[5]Institute of Inflammation and Repair, University of Manchester, Manchester, United Kingdom.

[6]Genes, Environment & Complex Disease, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[7]Centre for Social and Early Emotional Development, Faculty of Health, Deakin University, Burwood, Victoria, Australia.

[8]School of Population and Global Health, University of Melbourne, Parkville, Victoria, Australia

Search Strategies

Table S1 Search strategy for Medline (Ovid)

No.	Search Query
1.	exp Food Hypersensitivity/
2.	((nut*1 or peanut* or cashew* or tree-nut or egg*1 or milk or shellfish or shell-fish or wheat or food or almond*) adj3 (allerg* or hypersensiti* or hyper-sensiti* or sensiti*)).tw,kf,hw.
3.	exp Genome-Wide Association Study/
4.	(gene*1 or genetic* or mutation* or polymorphism* or SNP* or allel* or genome or genomewide or genome-wide or GWAS).tw,kf,hw.
5.	exp Genes/
6.	exp Genetic Predisposition to Disease/ or exp Polymorphism, Single Nucleotide/
7.	(newborn* or neonat* or infan* or toddler* or girl or girls or boy or boys or pre-schooler* or preschooler* or child or children or childhood or adolescen* or pediatric* or paediatric* or youth* or teen or teens or teenage*).af.
8.	ge.fs.
9.	(1 or 2) and (3 or 4 or 5 or 6 or 8) and 7
10.	Limit 9 to English language

Table S2 Search strategy for Embase (Ovid)

No.	Search Query
1.	exp food allergy/
2.	((nut*1 or peanut* or cashew* or tree-nut or egg*1 or milk or shellfish or shell-fish or wheat or food or almond*) adj3 (allergy or allergies or allergic*1 or hypersensiti* or hyper-sensiti* or sensiti*)).tw,kw,hw.
3.	(gene*1 or genetic* or mutation* or polymorphism* or SNP* or allel* or genome or genomewide or genome-wide or GWAS).tw,kw,hw.
4.	exp gene/
5.	exp genome-wide association study/
6.	exp genetic predisposition/
7.	exp single nucleotide polymorphism/
8.	(newborn* or neonat* or infan* or toddler* or girl or girls or boy or boys or pre-schooler* or preschooler* or child or children or childhood or adolescen* or pediatric* or paediatric* or youth* or teen or teens or teenage*).af.
9.	(1 or 2) and (3 or 4 or 5 or 6 or 7) and 8
10.	limit 9 to english language

PubMed search strategy

(“food allergy” OR “food allergies” OR “food sensitivity” OR “food sensitivities” OR “nut allergy” OR “nut allergies” OR “nut sensitivity” or “nut sensitivities” OR “peanut allergy” OR “peanut allergies” OR “peanut sensitivity” or “peanut sensitivities” OR “cashew allergy” OR “cashew allergies” OR “cashew sensitivity” OR “cashew sensitivities” OR “tree nut allergy” OR “tree nut allergies” OR “tree nut sensitivity” OR “tree nut sensitivities” OR “tree-nut allergy” OR “tree-nut allergies” OR “tree-nut sensitivity” OR “tree-nut sensitivities” OR “egg allergy” OR “egg allergies” OR “egg sensitivity” OR “egg sensitivities” OR “milk allergy” OR “milk allergies” OR “milk sensitivity” OR “milk sensitivities” OR “shellfish allergy” OR “shellfish allergies” OR “shellfish sensitivity” OR “shellfish sensitivities” OR “shell fish allergy” OR “shell fish allergies” OR “shell fish sensitivity” OR “shell fish sensitivities” OR “shell-fish allergy” OR “shell-fish allergies” OR “shell-fish sensitivity” OR “shell-fish sensitivities” OR “wheat allergy” OR “wheat allergies” OR “wheat sensitivity” OR “wheat sensitivities” OR “almond allergy” OR “almond allergies” OR “almond sensitivity” OR “almond sensitivities”) AND (gene OR genes OR genetic* OR mutation* OR polymorphism* OR SNP OR SNPs OR allel* OR genome OR genomewide OR genome-wide OR “genome wide” OR GWAS) AND (newborn* OR baby OR babies OR neonat* OR infan* OR toddler* OR pre-schooler* OR preschooler* OR kindergarten OR boy OR boys OR girl OR girls OR child OR children OR childhood OR adolescen* OR pediatric* OR paediatric* OR youth* OR teen OR teens OR teenage*) AND (NOTNLM OR publisher[sb] OR inprocess[sb] OR pubmednotmedline[sb] OR indatareview[sb] OR pubstatusaheadofprint)

Risk of Bias Assessment Criteria

In total, 9 criteria were used to assess risk of bias in eligible studies. These criteria were modified from those formulated by two previous studies (1, 2). Eligible studies were scored based on criteria encompassing study reproducibility, study design and appropriate statistical analyses. Each criteria were scored 1 if present or sufficiently meeting the criteria and 0 if absent. The score for each criteria were summed, giving a maximum total score ranging from 7 to 11 as some of the studies were not scored in some criteria. These scores were then transformed to a 10-point scale for comparison across studies. Studies were then ranked low if they scored 0 to 4, moderate for scores of 5 to 7 and high if they scored 8 or more. The criteria used were as follows:

Study reproducibility

1. Hardy-Weinberg Equilibrium (1 point)

Studies were scored for this criteria if they tested for Hardy–Weinberg equilibrium (HWE), and excluded and/or identified SNPs that did not pass HWE. Studies were considered adequate regardless of whether the cut-off used for HWE assessment was defined or otherwise.

2. Genotyping Quality Control (1 point)

Studies were considered adequate if they provided details on genotyping methods or included a reference to an article which did. Studies would have to provide information on one of the following measures of quality control: duplicate genotyping, blind genotyping (performed genotyping whilst blind to the clinical status of the patient) and/or process of analysing genotyping calls.

3. Polymorphisms/Primers (1 point)

Studies provided reference numbers (rs numbers) or primer sequences. If not, a reference to a previous publication which included these details was cited.

Study design

4. Power calculation (1 point)

Studies were scored if power calculation was done as part of the study whether retrospectively or prospectively.

5. Recruitment of cases (1 point)

Cases were representative of the population. Population-based studies and birth cohorts were scored as adequate but studies of hospital-based participants failed to obtain a point. For genome wide association studies (GWAS), only the discovery cohort was assessed for this component.

6. Recruitment of controls (2 points)

Controls were considered adequate if they were obtained from the same population as cases (1 point). Additional point was given if studies provided sufficient details that enable

ascertainment of non-atopic/unaffected status of the control group. For genome wide association studies (GWAS), only the discovery cohort was assessed for this component.

7. Quality of phenotype (1 point)

Acceptable studies scored 1 point if oral food challenge (OFC) was used to define food allergy. Studies which used a combination of classifications including OFC were not allocated a point. For instance, in some studies, certain participants were defined as food allergic based on OFC where available while others were ascertained by history of reaction in conjunction with measures of IgE sensitisation. These studies did not

Statistical analyses

8. Multiple testing adjustment (1 point)

Acceptable studies included multiple testing adjustments when two or more SNPs were studied. This category was marked not applicable if only a single polymorphism was analysed or if the study analysed SNPs as a binary variable, e.g. multiple SNPs at the same locus combined into 'has mutation' vs 'no mutation'.

9. Population stratification (1 point)

Population stratification were considered present if one of the following conditions were met:

- Statistical adjustment for population stratification was made AND/OR
- An independent set of polymorphic markers such as ancestry informative markers (AIMs) were genotyped AND/OR
- Presence of a population control group which was either genotyped or used as a comparison analyses AND/OR
- Analyses carried out only in one population excluding participants of other populations (in studies that were carried out in several populations)

Where these were absent, studies would need to have demonstrated an effort to gather a homogeneous population. Studies carried out in a homogenous population were not scored for this criteria. Studies that addressed population issues as a limitation were marked as 1 for this component. If studies did not address ethnicity or issues surrounding population and made no attempt to deal with stratification, they would fail to obtain a point for this criteria.

10. Replication (1 point)

Adequate studies included an independent replication cohort to validate findings observed in discovery cohort.

Table S3 Risk of bias assessment of included studies

Author	Study Reproducibility			Statistical analyses			Study Design				Study Quality
	Hardy-Weinberg Equilibrium	Genotyping Quality Control	Polymorphisms/Primers	Multiple testing adjustment	Population stratification	Replication	Power calculation	Recruitment of Cases	Recruitment of Controls	Phenotype definition	
Abe Jacob, 2013 (3)	1	0	1	1	0	0	1	0	2	0	Moderate
Amoli, 2002 (4)	0	0	1	NA	NA	0	0	0	0	0	Low
Asai, 2013 (5)	0	0	1	NA	1	0	0	1	0	0	Low
Asai, 2017 (6)	1	1	1	1	1	0	0	1	1	0	Moderate
Ashley, 2017 (7)	1	1	1	0	1	1	0	1	2	1	High
Ashley, 2017 (8)	1	1	1	1	1	1	1	1	2	1	High
Brown, 2011 (9)	0	0	1	NA	0	1	0	1	2	1	Moderate
Buyuktiryaki, 2016 (10)	0	0	1	0	0	0	0	0	2	1	Low
Campos, 2007 (11)	1	0	1	0	NA	0	1	0	1	0	Low
Chen, 2012 (12)	1	0	1	0	NA	0	0	0	2	0	Low
Dreskin, (13)	1	0	1	0	1	0	0	0	2	0	Moderate
Galli, 2010 (14)	0	0	1	0	0	0	0	0	1	1	Low
Gaudieri, 2012 (15)	1	0	1	0	NA	0	0	1	2	0	Moderate

Gawronska-Szklarz, 2001 (16)	0	0	0	0	NA	0	0	0	2	1	Low
Hand, 2004 (17)	0	0	1	1	1	0	0	0	0	0	Low
Hirota, 2017 (18)	0	0	1	0	NA	1	0	0	1	0	Low
Hitomi, 2009 (19)	1	0	1	0	NA	0	1	0	1	0	Low
Hong, 2015 (20)	1	1	1	1	1	1	0	1	2	0	High
Howell, 1998 (21)	0	0	1	1	NA	0	0	0	0	0	Low
Li, 2015 (22)	0	1	1	1	1	1	0	0	2	0	Moderate
Madore, 2013 (23)	0	1	1	1	NA	0	0	0	1	0	Low
Marenholz, 2017 (24)	1	1	1	1	1	1	1	0	1	0	High
Martino, 2017 (25)	1	1	1	1	1	1	0	1	2	0	High
Pawlik, 2004 (26)	1	0	1	0	NA	0	0	0	1	1	Low
Savilahti, 2010 (27)	0	0	1	1	0	0	0	1	2	1	Moderate
Shreffler, 2006 (28)	0	0	1	1	0	0	1	0	2	0	Moderate
Tamura, 2003 (29)	0	0	1	1	NA	0	0	0	1	0	Low
Tan, 2012 (30)	0	0	1	0	NA	0	0	1	2	0	Low

Venkataraman, 2014 (31)	1	1	0	NA	0	0	0	1	2	0	Moderate
Woo, 2003 (32)	1	0	1	NA	1	0	0	0	1	0	Low
Gene x Environment Interaction Studies											
Brough, 2014 (33)	0	0	1	NA	NA	0	0	1	2	0	Low
Koplin, 2016 (34)	1	1	1	0	1	0	0	1	2	1	Moderate

NA – not applicable. Criteria marked as NA were not scored for relevant studies as described in the online repository text.

Table S4 Summary of included studies

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
Abe Jacob, 2013 (3)	Brazil	Not mentioned	Cow's milk allergy	OFC or History of reaction		50 children, aged ≥ 5 years		224 individuals, age not provided		Candidate gene studies (no replication cohort)	IL10: (-3575A/T, -2849A/G, -2763A/C, -1082G/A, -592C/A) TGFb1: (-509C/T)	Moderate
Amoli, 2002 (4)	UK	Caucasian	Nut allergy	History of reaction + SPT		71 children with age of onset of nut allergy: 9.7 ± 12.1 years		184 blood donors, age not provided		Candidate gene studies (no replication cohort)	STAT6: 2964 G/A 3'UTR	Low
Asai, 2013 (5)	Canada	Caucasian	Peanut allergy	OFC or History of reaction + SPT/sIgE + or SPT + sIgE		679 subjects, 9.3 ± 4.0 years, range 0 -21 years (Age on 1st Jan 2009) (N=663 after QC)		894 adults, 65.5 ± 10.2, range 33-84 years (N=889 after QC) 268 newborns, 9.7 ± 0.5 years, 5range 7-10 years (N=267 after QC)		Candidate gene studies (no replication cohort)	FLG: rs61816761, rs41370446, rs138726443, rs150597413	Low

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	***Replication cohort***	***Discovery cohort***	***Replication cohort***	***Discovery cohort***	***Replication cohort***			
Asai, 2017 (8)[+]	Canada, Australia	Self-identified caucasian	Peanut and food allergy	OFC or History of reaction + SPT/sIgE or SPT + sIgE		987 cases, 12 ± 6 years, range: 1-63 years (N=850 after quality control)		987 controls, 49 ± 25 years, range 6-93 years (N= 926 after quality control)		GWAS	Genome-wide	Moderate
Ashley, 2017 (7)	Australia	<u>Discovery:</u> Caucasian, Asian and mixed Asian-Caucasian	Food allergy	OFC + SPT	OFC or History of reaction + SPT/sIgE or 95% PPV	367 food allergy cases 12.7 ± 0.75 months, 199 food sensitized but tolerant cases 12.6 ± 0.68 months	36 food allergic cases from the Barwon Infant Study (BIS), and 57 food allergic cases from the Melbourne Atopic Cohort (MACs), 72 food allergic cases from the Peanut Allergen Threshold Study (PATS) and 38 food	156 non-food allergic controls 12.6 ± 0.65 months	132 non-allergic controls (BIS) 198 non-allergic controls (MACS), 1.1±0.1 years	Candidate gene studies (with replication cohort)	IL13, nine tag SNPs	High

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
							allergic cases from the Probiotic and Peanut Oral Immuno-Therapy study (PPOIT), 4.1 ± 4.0 years					
Ashley, 2017 (8)	Australia	Discovery: Caucasian (n=503), Asian (n=74) or mixed Asian-Caucasian (n=145). Replication: 657 individuals to be of European descent, 217 of mixed European-Asian descent and	Food allergy	OFC + SPT	OFC or History of reaction + SPT/sIgE or 95% PPV	367 food-allergic, age at recruitment 12.7 ± 0.75 months	203 food-allergic children, 2.5 years	156 non-allergic controls, age at recruitment 12.6 ± 0.65 months	330 non-atopic controls, 2.5years	Candidate gene studies (with replication cohort)	SPINK5, Seventy-seven tag-SNPs	High

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
		32 of Asian descent.										
Brown, 2011 (9)	Canada, Ireland, Netherlands, UK	White	Peanut allergy	OFC	OFC or sIgE +/- SPT	71 cases, English: recruited at birth Dutch: range (mean) 3-14 years (7.5) Irish: range (mean) 1-18 (10.5)	390 cases, mean age 9.5 years (range 0-21 years)	1000 non-peanut sensitised controls recruited at birth 6851 population controls, no age provided	891 controls, mean age 57.5 years (range 23-77 years)	Candidate gene studies (with replication cohort)	FLG pooled English/ Dutch/Irish case collection: combined null genotype of 2 mutations (R501X and 2282del4)) Candian: 4 mutations (R501X, 2282del4, R2447X, and S3247X)	Moderate
Buyuktiryaki, 2016 (10)	Turkey	Not mentioned	Food allergy	OFC + History of reaction + SPT/sIgE		100 children, median (IQR): 3.2 (1.7–7.0) years (Genetic analyses on 90 children)		112 children, median (IQR): 4.0 (2.3–7.0) (Genetic analyses on 108 children)		Candidate gene studies (no replication cohort)	IDO1 and IDO2 genes: 10 SNPs, rs3808606, rs3824259, rs10089084, rs6991530, rs10504013 rs11992749, rs10109853, rs4503083, rs2955903, rs7820268	Low
Campos, 2007 (11)	Japan	Japanese	Food allergy	OFC or History of reaction		88 childhood patients, 7.1 ± 5.0 years		101 children age, 9.45 ± 1.5 years		Candidate gene studies (no replication cohort)	CD14:-159 and -550 polymorphisms	Low

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
Chen, 2012 (12)+	Taiwan	Taiwanese	Food allergy	History of reaction + SPT/sIgE		37 patients, 8.06 ± 6.25 years (range 1-32 years)		52 controls, 17.85 ± 15.19 years (range 1-59 years)		Candidate gene studies (no replication cohort)	IL10: rs1800896 and rs1800872	Low
Dreskin, 2011 (13)+	USA	All ethnicities, predominantly European descent (54/64)	Severe peanut allergy	OFC or sIgE or History of reaction + SPT		53 highly peanut allergic individuals, range 3-69 years		64 peanut-tolerant full-siblings, age not provided		Candidate gene studies (no replication cohort)	CD14: rs2569190 and rs2569193	Moderate
Galli, 2010 (14)	Italy	Not mentioned	Cow's milk allergy	OFC + SPT		159 children (102 with eczema and 57 with food allergy), ranging from 6-198 months,		147 healthy controls, matched for age and sex		Candidate gene studies (no replication cohort)	TLR-2 R753Q (rs5743708) and TLR-4 D299G (rs4986790	Low

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
						median 58 months						
Gaudieri, 2012 (15)	Australia	Caucasian	Food allergy and other allergy	History of reaction/doctor-diagnosis + SPT		35 allergic infants (cohort 1) + 30 children with FA (cohort 2), followed from birth to age 5 years		35 non-allergic infants, followed from birth to age 5 years		Candidate gene studies (no replication cohort)	tagSNP rs12979860 upstream of IL28B. 4 additional ones in LD (rs8403219, rs28416813, rs8103142 and rs4803217). Another tagging SNP rs8099917 10 kb upstream of IL28B	Moderate
Gawronska-Szklarz, 2001 (16)	Poland	White	Food allergy and other allergy	OFC + SPT + sIgE		136 children (61 with FA and other atopy, 75 with FA only), range 1.5 to 17 years		123 healthy children, range 2 to 17 years		Candidate gene studies (no replication cohort)	N-acetyltransferase 2 (NAT2) NAT2*4 allele, NAT2*5, NAT2*6, and NAT2*7	Low
Hand, 2004 (17)[+]	UK	Majority Caucasian, some mixed race	Nut allergy	History of reaction + SPT		84 unrelated patients ranging from 3-56 years		1798 HLA typed random blood donors served as a population frequency control.		Candidate gene studies (no replication cohort)	Polymorphisms within the HLA-A, B, DRB1, DQB1 loci	Low

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
								Atopic control group of 82 random subjects with age range 16–61 years				
Hirota, 2017 (18)	Japan	Japanese	Food allergy	OFC or history of reaction	OFC or history of reaction	593 FA cases, 5.2±3.7 years	279 cases, 5.8±3.3 years	985 controls, 50.0±9.2 years	886 controls, 50.6±12.9 years	Candidate gene studies (with replication cohort)	19 and 7 susceptibility variants previously reported in GWAS for AD and EoE, respectively. FLG, C11orf30/LRRC32, TMEM232/SLC25A46, TNFRSF6B/ZGPAT, OVOL1, ACTL9,KIF3A/IL13,IL1RL1/IL18R1/IL18RAP,GLB1,CCDC80,GPSM3 (MHC region),CARD11,ZNF365,OR10A3/NLRP10,CYP24A1/PFDN4,IL2/IL21,PRR5L,CLEC16A/DEXI ZNF652, TSLP/WDR36,CAPN14,XKR6,LOC283710/	Low

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
											KLF13,C11orf30, STAT6, ANKRD27 Additional, 6 FLG null variants, c.3321delA, p.Q1701*, p.S2554*, p.S2889*, p.S3296*, and p.K4022*,	
Hitomi, 2009 (19)	Japan	Japanese	Food-induced anaphylaxis, FA, AIA	OFC or History of reaction + sIgE		320 pediatric patients, 4.2 ± 3.3 years		254 children, 9.0 ± 1.7 years		Candidate gene studies (no replication cohort)	15 Tag SNPs of the exons and introns of NLRP3 with a minor allele frequency of greater than 10% in the HapMap Japanese data set rs2027432 rs12079994 rs4925648 rs4925650 rs12048215 rs10754555 rs3806265 rs10925019 rs4925654 rs4612666 rs10925026 rs12565738 rs4378247 rs10754558 rs10733112	Low
Hong, 2015 (20)	USA	European and non-European	Food Allergy, including peanut, egg	History of reaction + SPT/sIgE	History of reaction + SPT/sIgE	671 European food allergic cases, 155 non-	62 PA European cases, 24 PA non-European	144 non-allergic non-sensitized normal controls and	69 European controls, 58 non-European controls,	GWAS	Genome-wide	High

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
			and milk allergy			European food allergic children. Children between 0-21 years old, parents information not available.	cases, ranging from 0-21 years	1,382 European controls of uncertain phenotypes (234 children and 1,148 parents). Children between 0-21 years old, parents information not available.	ranging from 0-21 years			
Howell, 1998 (21)	UK	Cases all Caucasians	Peanut allergy	History of reaction + SPT		Study group: 34 nuclear families each containing one peanut allergic proband, three nuclear families each containing two peanut		293 bone marrow and cadaveric renal donors, age not provided		Candidate gene studies (no replication cohort)	HLA class II DRB1, DQB1 and DPB1	Low

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
						allergic siblings - 161 individuals: contained 50 peanut allergic individuals mean age 5 years, 34 non-peanut allergic siblings (of whom 12 were non-atopic and 22 atopic) plus non-peanut allergic parents						
Li, 2015 (22)	USA	European and African American	Food allergy	History of reaction + SPT/sIgE	History of reaction + SPT/sIgE	357 cases, 5.6 ± 4.4 years	167 cases, 5.4 ± 3.9 years	3980 controls, 10.2 ± 5.4 years	1573 controls, 12.6 ± 3.8 years	GWAS	Genome-wide	Moderate
Madore, 2013 (23)	Canada	Self-reported Caucasian	Peanut allergy	History of reaction + SPT/sIgE or		590 children. Post-QC: 311		332 children. Post-QC: 226		Candidate gene studies (no replication cohort)	HLA DQB1	Low

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
				SPT + sIgE or OFC		children, 11 ± 4 years		subjects, 4 ± 4 years				
Marenholz, 2017 (24)	Germany, USA	European	Food allergy	OFC or History of reaction + sIgE	History of reaction + SPT/sIgE or OFC	523 children (n=497 after QC) Mean food allergy diagnosis 2.1 years	Replication 1: 380 German cases, 2.8 years mean food allergy diagnosis (n=379 after QC) Replication 2: 671 FA children, age not provided	2682 population-based, age not provided (N=2387 after QC)	Replication 1: 986 controls (n=984 after QC) Replication 2: 1526 controls Age not provided	GWAS	Genome-wide	High
Martino, 2017 (25)	Australia	European, Asian, mixed	Peanut allergy	OFC or recent reaction	OFC or recent reaction	73 infants, 12.8 ± 0.80 months	117 peanut allergic cases from various studies, age not provided	148 infants, 12.6 ± 0.67 months	380 non-allergic controls from HealthNuts and the Barwon Infant Study (BIS), age not provided	GWAS	Genome-wide	High

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
Pawlik, 2004 (26)	Poland	Caucasians and came from the Pomeranian region of Poland	Atopic disease including food allergy	OFC + SPT + sIgE		217 children (77 with food allergy) ranging from 1.5–17 years		124 healthy subjects ranging from 5–17 years		Candidate gene studies (no replication cohort)	FcyRIIa	Low
Savilahti, 2010 (27)	Finland	Not mentioned	Cow's milk allergy	OFC + SPT/sIgE		87 patients with CMA (67 IgE-mediated mean age 8.6 years, range 8.0-9.1 years and 20 non-IgE mediated mean 8.5 years, range 8–9 years		76 control subjects ranging from 8.1–9.3 years (mean 8.6 years)		Candidate gene studies (no replication cohort)	Panel of HLA class II haplotypes, PTPN22 R620W allele (rs2476601) and 5 filaggrin null mutations (del22824, 501-C/T, R2447X, S3247, 3702delG)	Moderate
Shreffler, 2006 (28)	USA	69 White, 4 Asian	Peanut allergy	History of reaction + sIgE		73 probands, mean age 6.5 years		75 siblings, mean age 8 years		Candidate gene studies (no replication cohort)	HLA DR and DQ: 10 individual DQB1 alleles, 65 allele groups DR	Moderate
Tamura, 2003 (29)	Japan	Japanese	Food-related anaphylaxis	History of reaction + sIgE		102 children with a major allergic disease including		66 control subjects, age not provided		Candidate gene studies (no replication cohort)	STAT6: G2964A variant	Low

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
						atopic dermatitis, bronchial asthma, and/or food-related anaphylaxis. 71 children had AD, 47 had BA, and 14 had FA, age not provided						
Tan, 2012 (30)	Australia	Caucasian	Food allergy	OFC or SPT/sIgE or History of reaction		321 one-year-old infants		126 one-year-old infants		Candidate gene studies (no replication cohort)	FLG: R501X, 2282del4, R2447X, S3247X and 3702delG	Low
Venkataraman, 2014 (31)	UK	Not mentioned	Food allergy	History of reaction +/- SPT		1374 children aged 1 year 1231 children aged 2 years 1218 children aged 4 years 1373 children aged 10				Candidate gene studies (no replication cohort)	FLG: R501X, 2282del4, S3247X, 3702delG and R2447X	Moderate

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
						years 1313 children aged 18 years 1150 18-year-old children genotyped						
Woo, 2003 (32)[+]	USA	White and mixed	Food allergy	OFC or History of reaction + SPT/sIgE		77 patients 5.2 ± 5.3 years (range 0.8-33.9 years)		61 non-atopic, non-asthmatic control adults 29.9 ± 7.4 years (range 20-51 years)		Candidate gene studies (no replication cohort)	CD14: –159 C→T	Low
Brough, 2014 (33)	UK	FLG genotype: only white, other analysis non-white inclusive	Peanut allergy	OFC or History of reaction +SPT/sIgE		20 children with peanut allergy at age 8, 11 years or both		577 children without peanut allergy at age 8, 11 years or both		Candidate gene studies (no replication cohort)	FLG R501X, S3247X, R2447X. 2282del4, 3673delC and 3702delG	Low

Author	Country	Ethnicity	Type of allergy	Definition of Cases		Participants - Cases (sample size, age mean ± SD)		Participants - Controls (sample size, age mean ± SD)		Type of genetic study	Genes/SNPs of interest	Quality Score
				Discovery cohort	*Replication cohort*	*Discovery cohort*	*Replication cohort*	*Discovery cohort*	*Replication cohort*			
Koplin, 2016 (34)	Australia	Caucasian and Asian	Food Allergy	OFC + SPT/sIgE		338 food-allergic at age 1 55 with persistent egg allergy at age 2		269 non-allergic at age 1 50 with resolved egg allergy at age 2		Candidate gene studies (no replication cohort)	GC: rs7041 and rs4588	Moderate

[+] Studies carried out in participants across ages including children

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Appendix 7 MAF of SNPs genotyped

Table 1 List of 37 SNPs genotyped

					1000G EAS		1000G EUR			
SNP	**CHR**	**Gene annotated to in literature**	**Reference allele**	**Alternate allele**	**MAF reference allele**	**MAF alternate allele**	**MAF reference allele**	**MAF alternate allele**	**GWAS[a]**	**Investigated outcome in literature**
rs1056204	2	*ADAM17*	A	C	0.997	0.003	0.68	0.32	Genotyped	Helminth diversity (141)
rs10495562	2	*ADAM17*	T	C	0.979	0.021	0.489	0.511	Genotyped	Helminth diversity (141)
rs7579207	2	*DPP10*	A	G	0.023	0.977	0.306	0.694	Genotyped	Helminth diversity, allergy (141)
rs231735	2	*CTLA4*	G	T	0.267	0.733	0.516	0.484	Imputed	Helminth diversity, allergy (141)
rs11571291	2	*CTLA4*	T	C	0.732	0.268	0.555	0.445	Imputed	Helminth diversity, allergy (141)
rs231804	2	*CTLA4*	C	T	0.267	0.733	0.451	0.549	Imputed	Helminth diversity, allergy (141)
rs4353658	2	*DPP10*	G	A	0.854	0.146	0.55	0.45	Genotyped	Helminth diversity, allergy (141)
rs6749207	2	*EDAR*	T	C	0.192	0.808	0.855	0.145	Imputed	Helminth diversity, hair thickness (141)

					1000G EAS		1000G EUR			
SNP	**CHR**	**Gene annotated to in literature**	**Reference allele**	**Alternate allele**	**MAF reference allele**	**MAF alternate allele**	**MAF reference allele**	**MAF alternate allele**	**GWAS[a]**	**Investigated outcome in literature**
rs12619285	2	*IKZF2*	A	G	0.367	0.633	0.746	0.254	Imputed	Helminth diversity, allergy (141)
rs4368333	2	*KCNS3*	C	A	0.945	0.055	0.432	0.568	Imputed	Helminth diversity, allergy (141)
rs6737848	2	*SOCS5*	C	G	0.594	0.406	0.926	0.074	Imputed	Atopic bronchial asthma (317)
rs4684083	3	*CHL1*	T	C	0.951	0.049	0.745	0.255	Genotyped	Helminth diversity (141)
rs4682429	3	*CD200R1L*	G	A	0.901	0.099	0.558	0.442	Genotyped	Helminth diversity (141)
rs10004195	4	*IL4R*	T	A	0.519	0.481	0.721	0.279	Imputed	Self reported allergy and Helicobacter pylori serologic status (318)
rs10024216	4	*IL4R*	G	A	0.433	0.567	0.608	0.392	Imputed	Self reported allergy and Helicobacter pylori serologic status (318)
rs10014145	4	*SLC39A8*	A	G	0.876	0.124	0.675	0.325	Genotyped	Helminth diversity (141)
rs877741	5	*ADRB2*	T	C	0.215	0.785	0.812	0.188	Genotyped	Helminth diversity, allergy (141)

					1000G EAS		1000G EUR			
SNP	**CHR**	**Gene annotated to in literature**	**Reference allele**	**Alternate allele**	**MAF reference allele**	**MAF alternate allele**	**MAF reference allele**	**MAF alternate allele**	**GWAS[a]**	**Investigated outcome in literature**
rs2243290	5	*IL4*	C	A	0.217	0.783	0.83	0.17	Genotyped	Helminth diversity (141)
rs2243268	5	*IL4*	A	C	0.224	0.776	0.833	0.167	Genotyped	Helminth diversity, allergy (141)
rs12186803	5	*KIF3A/IL4*	G	A	0.247	0.753	0.831	0.169	Imputed	Helminth diversity (141)
rs2243250	5	*IL4*	C	T	0.221	0.779	0.832	0.168	Imputed	Th2-predominant immune response (319)
rs2070874	5	*IL4*	C	T	0.221	0.779	0.832	0.168	Genotyped	Helminth diversity (141)
rs7192	6	*HLA*	T	G	0.313	0.687	0.367	0.633	Genotyped	Peanut allergy (118)
rs10237930	7	*NPSR1*	T	C	0.731	0.269	0.504	0.496	Imputed	Helminth diversity, allergy (141)
rs7849955	9	*TLR4*	G	A	0.998	0.002	0.854	0.146	Imputed	Helminth diversity, allergy (141)
rs1930713	9	*TLR4*	G	A	0.957	0.043	0.736	0.264	Genotyped	Helminth diversity, allergy (141)
rs2245960	9	*TLR4*	C	T	0.956	0.044	0.768	0.232	Imputed	Helminth diversity, allergy (141)
rs1952692	9	*TYRP1*	A	C	0.954	0.046	0.668	0.332	Imputed	Helminth diversity (141)
rs10905349	10	*GATA3*	A	G	0.525	0.475	0.165	0.835	Genotyped	Helminth diversity, allergy (141)

					1000G EAS		1000G EUR			
SNP	**CHR**	**Gene annotated to in literature**	**Reference allele**	**Alternate allele**	**MAF reference allele**	**MAF alternate allele**	**MAF reference allele**	**MAF alternate allele**	**GWAS[a]**	**Investigated outcome in literature**
rs2069705	12	*IFNG*	G	A	0.757	0.243	0.327	0.673	Imputed	Atopic bronchial asthma (317)
rs2289046	13	*IRS2*	T	C	0.535	0.465	0.69	0.31	Imputed	Total IgE levels in asthmatics (316)
rs1805388	13	*LIG4*	G	A	0.79	0.21	0.837	0.163	Imputed	IgE levels to *Ascaris* (316)
rs7329078	13	*PHF11*	T	C	0.192	0.808	0.403	0.597	Imputed	Helminth diversity, allergy (141)
rs708491	14	*PTGER2*	A	G	0.993	0.007	0.72	0.28	Genotyped	Helminth diversity, allergy (141)
rs16967593	17	*STAT5B*	T	A	0.636	0.364	0.719	0.281	Imputed	Atopic bronchial asthma (317)
rs1800469	19	*TGFb1*	A	G	0.547	0.453	0.312	0.688	Genotyped	Helminths infections, allergy (320)
rs1800470	19	*TGFb1*	G	A	0.555	0.445	0.382	0.618	Imputed	Helminths infections, allergy (320)
rs2241712	19	*TGFb1*	C	T	0.554	0.446	0.325	0.675	Imputed	Helminths infections, allergy (320)

Single nucleotide polymorphism (SNP); Chromosome (CHR); Minor allele frequency (MAF); East Asian population (EAS), European population (EUR), 1000 genomes project (1000G); Genome wide association studies (GWAS)

[a] Column refers to whether genotype data for SNP extracted from HealthNuts GWAS was obtained via SNP array imputation or direct genotyping of samples

Appendix 8 Primer sequences

Table 1 Primers used for genotyping assay for association analyses with food allergy in Chapter 7

Name	Sequence	Scale	Purification
rs1800469_W1_F	ACGTTGGATGAGGGTGTCAGTGG-GAGGAG	25nm	STD
rs12186803_W1_F	ACGTTGGATGAAACTCAG-GACCCGAAGGC	25nm	STD
rs1805388_W1_F	ACGTTGGATGCACAAATCTG-CAAAAGGAACG	25nm	STD
rs2245960_W1_F	ACGTTGGATGTATGGA-GAGCCTTAAAAGCG	25nm	STD
rs6749207_W1_F	ACGTTGGATGCTAGATAATATAA-GCTCCC	25nm	STD
rs2241712_W1_F	ACGTTGGATGAGCGCAAAA-GACCCGCCTT	25nm	STD
rs6737848_W1_F	ACGTTGGATGCATTTCTG-CAAAAAGGTGTT	25nm	STD
rs10014145_W1_F	ACGTTGGATGATCACAC-CTAATGATCAGGG	25nm	STD
rs1952692_W1_F	ACGTTGGATGTTCACTGGAGGC-TACCTATC	25nm	STD
rs12619285_W1_F	ACGTTGGATGTCATAGCAC-CTCAGCTTACC	25nm	STD
rs231735_W1_F	ACGTTGGATGTGCCTAC-CACAAGGATTGCT	25nm	STD
rs16967593_W1_F	ACGTTGGATGCTGGGAGTAGATA-CACTGTC	25nm	STD
rs10905349_W1_F	ACGTTGGATGGATGGCTGAT-TCCTTCCTTG	25nm	STD
rs877741_W1_F	ACGTTGGATGTGTGTTAGTGGTG-CAGAGTG	25nm	STD
rs4682429_W1_F	ACGTTGGATGATCTGTAAGCGAG-GATGGAC	25nm	STD
rs11571291_W1_F	ACGTT-GGATGTCATGAAGGGAAAATACAC	25nm	STD
rs2289046_W1_F	ACGTTGGATGATCCCCTTCCCAAA-GCCCTT	25nm	STD
rs2243290_W1_F	ACGTTGGATGTGATCAAGTAGA-CAGGCAGG	25nm	STD
rs2070874_W1_F	ACGTTGGATGTG-CATCGTTAGCTTCTCCTG	25nm	STD
rs10495562_W1_F	ACGTTGGATGACAGCAA-GAAGGTCATTCCC	25nm	STD
rs1056204_W1_F	ACGTTGGATGTTTCTGCC-TACACATCCCTG	25nm	STD

Name	Sequence	Scale	Purification
rs2243268_W1_F	ACGTTGGATGACCACTGTG-CAATGCGTTTC	25nm	STD
rs4684083_W1_F	ACGTTGGATGGATTT-GAAGGCTTCCGTTCG	25nm	STD
rs4368333_W1_F	ACGTT-GGATGCATGGAACTGGCCTCAATAG	25nm	STD
rs4353658_W1_F	ACGTTGGATGCAAACATT-GTCCTCAGTGGG	25nm	STD
rs2069705_W1_F	ACGTTGGATGGGGCAAACTTGAT-TCCTGAC	25nm	STD
rs708491_W1_F	ACGTTGGATGGCTTCATCAG-GACATGGTTC	25nm	STD
rs10024216_W2_F	ACGTTGGATGGAGTGAGAGCTGAA-GAAATG	25nm	STD
rs2243250_W2_F	ACGTTGGATGTGATACGAC-CTGTCCTTCTC	25nm	STD
rs1930713_W2_F	ACGTTGGATGATGCCTTGTCAA-GAAGCACC	25nm	STD
rs7849955_W2_F	ACGTTGGATGGGGAAGTGACTTT-GGAAGAG	25nm	STD
rs7192_W2_F	ACGTTGGATGCTGGTGGG-CATCATTATTGG	25nm	STD
rs231804_W2_F	ACGTTGGATGCGTTTAACCTTTTAG-GAGGG	25nm	STD
rs10237930_W2_F	ACGTTGGATGGCTGACTGGG-TATATTACTC	25nm	STD
rs7579207_W2_F	ACGTTGGATGAGCCTGAC-TATGCCCTTTTC	25nm	STD
rs10004195_W2_F	ACGTTGGATGCGCCCTCTTCTG-CATGCTA	25nm	STD
rs7329078_W2_F	ACGTT-GGATGCAGGGGTTATGTGAATTTTG	25nm	STD
rs1800469_W1_R	ACGTTGGATGAGGAGAG-CAATTCTTACAGG	25nm	STD
rs12186803_W1_R	ACGTTGGATGGGAA-GCCATATTCCAGCTTT	25nm	STD
rs1805388_W1_R	ACGTTGGATGTT-GATGGCTGCCTCACAAAC	25nm	STD
rs2245960_W1_R	ACGTTGGATGGGCACCTGCTATA-TAGTCCA	25nm	STD
rs6749207_W1_R	ACGTTGGATGAGTCTGAACTCGG-CATGAGC	25nm	STD
rs2241712_W1_R	ACGTTGGATGATAAC-GCATGCGCCTTATGG	25nm	STD
rs6737848_W1_R	ACGTTGGATGATTCAAGGACAA-TAAGGAG	25nm	STD
rs10014145_W1_R	ACGTTGGATGAGTACTAA-GAGGCATCCCAC	25nm	STD
rs1952692_W1_R	ACGTTGGATGTGTAAAGTTT-GCCGAGGTGC	25nm	STD

Name	Sequence	Scale	Purification
rs12619285_W1_R	ACGTT-GGATGGAGCCCTAATCATGTTGCAG	25nm	STD
rs231735_W1_R	ACGTTGGATGGCTGATTTAGGGTG-GACTTC	25nm	STD
rs16967593_W1_R	ACGTTGGATGCACTGTCAC-TCTTCCGTTTC	25nm	STD
rs10905349_W1_R	ACGTTGGATGTCCAGATTCAG-CAAGGCAAG	25nm	STD
rs877741_W1_R	ACGTTGGATGGAAATTAA-GCAGCCTGCCTC	25nm	STD
rs4682429_W1_R	ACGTTGGATGCAC-CTTCTGTTCCAGATGGG	25nm	STD
rs11571291_W1_R	ACGTTGGATGCCTGAGAGATAA-GAAGGCAC	25nm	STD
rs2289046_W1_R	ACGTTGGATGATGTAACTGAA-GCAACCTAC	25nm	STD
rs2243290_W1_R	ACGTTGGATGTT-GGCTTCCTTCACAGGACA	25nm	STD
rs2070874_W1_R	ACGTTGGATGGAGGTGA-GACCCATTAATAG	25nm	STD
rs10495562_W1_R	ACGTTGGATGCAGTTAGTGTTAG-GAATAGCC	25nm	STD
rs1056204_W1_R	ACGTTGGATGTAGGAAGGTTT-GCTGAACGG	25nm	STD
rs2243268_W1_R	ACGTTGGATGATTTCCTAA-GCCCTTCGGTG	25nm	STD
rs4684083_W1_R	ACGTTGGATGTTGTAGTAGAGTAC-CAGCAG	25nm	STD
rs4368333_W1_R	ACGTTGGATGTTT-GCTTCTGGGTGAAGGAG	25nm	STD
rs4353658_W1_R	ACGTTGGATGGGTCAAATGCTCCTT-GCAAC	25nm	STD
rs2069705_W1_R	ACGTTGGATGAGGAGACTGAG-TCATAGAAG	25nm	STD
rs708491_W1_R	ACGTTGGATGCATGGCCATGGAC-GAAATAC	25nm	STD
rs10024216_W2_R	ACGTTGGATGTGTCAACCTCTCAG-CATGCC	25nm	STD
rs2243250_W2_R	ACGTT-GGATGTAACAGGCAGACTCTCCTAC	25nm	STD
rs1930713_W2_R	ACGTTGGATGACTCACTGCTGA-GAGGTAAG	25nm	STD
rs7849955_W2_R	ACGTTGGATGAC-CAGAACAGGTGTTACAGC	25nm	STD
rs7192_W2_R	ACGTTGGATGTCAC-CTCCATGTGCCTTACA	25nm	STD
rs231804_W2_R	ACGTTGGATGAACCAGCACAC-TCAAGTGTC	25nm	STD
rs10237930_W2_R	ACGTTGGATGCTTCAGAAAAC-TATGCTAGG	25nm	STD

Name	Sequence	Scale	Purification
rs7579207_W2_R	ACGTTGGATGAATGCTCACGTG-GAGCTATG	25nm	STD
rs10004195_W2_R	ACGTTGGATGCTCCTATGTTATGTG-TATTTG	25nm	STD
rs7329078_W2_R	ACGTTGGATGGCTGTCCTAACATT-GATCTC	25nm	STD
rs1800469_W1_UEP	CTGACCCTTCCATCC	100nm	STD
rs12186803_W1_UEP	AGGCTCCCTAAGTCA	100nm	STD
rs1805388_W1_UEP	ACGTGAGATGCAACA	100nm	STD
rs2245960_W1_UEP	TCCAAGAGTGCTGGA	100nm	STD
rs6749207_W1_UEP	GGCATGAGCAGGGTAG	100nm	STD
rs2241712_W1_UEP	GGAAGCGGGGTGGCTG	100nm	STD
rs6737848_W1_UEP	TTTTCAGTTGGCACCTT	100nm	STD
rs10014145_W1_UEP	ACTGAATTCCCTGAGTT	100nm	STD
rs1952692_W1_UEP	TGCCGAGGTGCTGATAA	100nm	STD
rs12619285_W1_UEP	AACACACTCACTATGAGA	100nm	STD
rs231735_W1_UEP	GGACTTCACTCATATCAGA	100nm	STD
rs16967593_W1_UEP	CCAAAGTAAGATGCAGACG	100nm	STD
rs10905349_W1_UEP	CAGCAAGGCAAGTGGAAAA	100nm	STD
rs877741_W1_UEP	GGTTCCTTCTCTTCCTATTA	100nm	STD
rs4682429_W1_UEP	GGGCCTCACAATAGTTACAA	100nm	STD
rs11571291_W1_UEP	TGAGAGTTGAAAGTAAGAGA	100nm	STD
rs2289046_W1_UEP	GCAACCTACTTTTGAAAATCA	100nm	STD
rs2243290_W1_UEP	CAGGACAGGAATTCTGCAAAA	100nm	STD
rs2070874_W1_UEP	GCTTCTCCTGATAAACTAATTG	100nm	STD
rs10495562_W1_UEP	GTTAGGAATAGCCTGTGATGAA	100nm	STD
rs1056204_W1_UEP	CAGCTTCTGTTTGCATCCTTGTC	100nm	STD
rs2243268_W1_UEP	CCCTTCGGTGGTATTAGAGAACA	100nm	STD
rs4684083_W1_UEP	cGGACTCAGGACAATAAAAATAA	100nm	STD
rs4368333_W1_UEP	ACTGGCCTCAATAGGATTTGTGATC	100nm	STD
rs4353658_W1_UEP	CAAATGCTCCTTGCAACACAAA-TACT	100nm	STD
rs2069705_W1_UEP	CTATCTAGCTATATGATTGTGAG-TTA	100nm	STD
rs708491_W1_UEP	AATACTTTCTACTA-TCGCCTCTGCTTCA	100nm	STD
rs10024216_W2_UEP	TCACCGAGTTGCTCA	100nm	STD
rs2243250_W2_UEP	ACTTGGGAGAACATTGT	100nm	STD
rs1930713_W2_UEP	TGAGAGGTAAGGCATCT	100nm	STD
rs7849955_W2_UEP	ACTTTGGAAGAGACATGC	100nm	STD
rs7192_W2_UEP	CATCTTCATCATCAAGGGA	100nm	STD
rs231804_W2_UEP	TTTTAGGAGGGTTTTGTTG	100nm	STD
rs10237930_W2_UEP	TCAATTTCCTCATATGTGAAA	100nm	STD
rs7579207_W2_UEP	TTCCAAAAATAATAGGAAGTCA	100nm	STD

Name	Sequence	Scale	Purification
rs10004195_W2_UEP	TTCATTATAATCTTAGCACTTTTT	100nm	STD
rs7329078_W2_UEP	GATCTCATTATTCAAGATATTGTG	100nm	STD

Appendix 9 Gene names

Table 1 Gene abbreviations and their corresponding description used in this thesis

Gene symbol	Description
ABCB11	ATP Binding Cassette Subfamily B Member 11
ACOT11	Acyl-CoA Thioesterase 11
ADAM17	ADAM Metallopeptidase Domain 17
ADRB2	Adrenoceptor Beta 2
ARHGAP24	Rho GTPase Activating Protein 24
ATP10A	ATPase Phospholipid Transporting 10A (Putative)
BCAS1	Breast Carcinoma Amplified Sequence 1
C11orf30	Chromosome 11 open reading frame 30
CCDC80	Coiled-Coil Domain Containing 80
CD14	CD14 Molecule
CD200R1L	CD200 Receptor 1 Like
CHCHD3	Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 3
CHIA	Chitinase Acidic
CHL1	Cell Adhesion Molecule L1 Like
CLEC16A	C-Type Lectin Domain Containing 16A
COG7	Component Of Oligomeric Golgi Complex 7
CTLA4	Cytotoxic T-Lymphocyte Associated Protein 4
CTNNA3	Catenin Alpha 3
DEXI	Dexi Homolog
DPP10	Dipeptidyl Peptidase Like 10
EDAR	Ectodysplasin A Receptor
EMCN	Endomucin
ERCC4	ERCC Excision Repair 4, Endonuclease Catalytic Subunit
ETAA1	ETAA1 Activator Of ATR Kinase
EXOC4	Exocyst Complex Component 4
FAM117A	Family With Sequence Similarity 117 Member A
FcyRIIa	Fc Fragment Of IgG Receptor IIa
FLG	Filaggrin
FLG-AS1	FLG Antisense RNA 1
FOXP3	Forkhead Box P3
FXR1	FMR1 Autosomal Homolog 1
GATA3	GATA Binding Protein 3
GC	GC Vitamin D Binding Protein
GLB1	Galactosidase Beta 1
HLA	Major Histocompatibility Complex

Gene symbol	Description
HLA- DQB1	Major Histocompatibility Complex, Class II, DQ Beta 1
HLA-A	Major Histocompatibility Complex, Class I, A
HLA-B	Major Histocompatibility Complex, Class I, B
HLA-DQA2	Major Histocompatibility Complex, Class II, DQ Alpha 2
HLA-DQB1	Major Histocompatibility Complex, Class II, DQ Beta 1
HLA-DRA	Major Histocompatibility Complex, Class II, DR Alpha
HLA-DRB1	Major Histocompatibility Complex, Class II, DR Beta 1
HMGA2	High Mobility Group AT-Hook 2
IDO1	Indoleamine 2,3-Dioxygenase 1
IDO2	Indoleamine 2,3-Dioxygenase 2
IER5L	Immediate Early Response 5 Like
IFNG	Interferon Gamma
IKZF2	IKAROS Family Zinc Finger 2
IL10	nterleukin 10
IL10RA	Interleukin 10 Receptor Subunit Alpha
IL13	Interleukin 13
IL2	Interleukin 2
IL21	Interleukin 21
IL26	Interleukin 26
IL28B/IFNL3	Interferon lambda 3
IL4	Interleukin 4
IL4R	Interleukin 4 Receptor
IL6	Interleukin 6
IMPAD1	Inositol Monophosphatase Domain Containing 1
IRS2	Insulin Receptor Substrate 2
ITIH5L	Inter-alpha inhibitor H5-like
KCNS3	Potassium Voltage-Gated Channel Modifier Subfamily S Member 3
KIF3A	Kinesin Family Member 3A
LIG4	DNA Ligase 4
LINGO2	Leucine Rich Repeat And Ig Domain Containing 2
LLPH	LLP Homolog, Long-Term Synaptic Facilitation Factor
LMX1A	LIM Homeobox Transcription Factor 1 Alpha
LRRC32	Leucine Rich Repeat Containing 32
LSP1	Lymphocyte Specific Protein 1
LUZP2	Leucine Zipper Protein 2
MACROD2	Mono-ADP Ribosylhydrolase 2
MDN1	Midasin AAA ATPase 1
NAT2	N-Acetyltransferase 2
NAV2	Neuron Navigator 2

Gene symbol	Description
NLRP10	NLR Family Pyrin Domain Containing 10
NLRP3	NLR Family Pyrin Domain Containing 3
NOVA1	NOVA Alternative Splicing Regulator 1
NPSR1	Neuropeptide S Receptor 1
ODZ/TENM3	Teneurin Transmembrane Protein 3
OR10A3	Olfactory Receptor Family 10 Subfamily A Member 3
OVOL1	Ovo Like Transcriptional Repressor 1
PAFAH1B1	Platelet Activating Factor Acetylhydrolase 1b Regulatory Subunit 1
PAX2	Paired Box 2
PHF11	PHD Finger Protein 11
PLAGL1	PLAG1 Like Zinc Finger 1
PTGER2	Prostaglandin E Receptor 2
PTPN22	Protein Tyrosine Phosphatase Non-Receptor Type 22
PYROXD1	Pyridine Nucleotide-Disulphide Oxidoreductase Domain 1
RBFOX1	RNA Binding Fox-1 Homolog 1
RGS21	Regulator Of G Protein Signaling 21
RHOBTB1	Rho Related BTB Domain Containing 1
RIMS2	Regulating Synaptic Membrane Exocytosis 2
RNF130	Ring Finger Protein 130
SALL3	Spalt Like Transcription Factor 3
SERPINB2	Serpin Family B Member 2
SERPINB7	Serpin Family B Member 7
SGCD	Sarcoglycan Delta
SKAP1	Src Kinase Associated Phosphoprotein 1
SLC25A46	Solute Carrier Family 25 Member 46
SLC2A9	Solute Carrier Family 2 Member 9
SLC39A10	Solute Carrier Family 39 Member 10
SLC39A8	Solute Carrier Family 39 Member 8
SOCS5	Suppressor Of Cytokine Signaling 5
SORBS2	Sorbin And SH3 Domain Containing 2
SPINK5	Serine Peptidase Inhibitor, Kazal Type 5
SSBP3	Single Stranded DNA Binding Protein 3
STAT5B	Signal Transducer And Activator Of Transcription 5B
STAT6	Signal Transducer And Activator Of Transcription 6
STXBP6	Syntaxin Binding Protein 6
SV2C	Synaptic Vesicle Glycoprotein 2C
TES	Testin LIM Domain Protein
TGFb1	Transforming Growth Factor Beta 1
TLR2	Toll Like Receptor 2

Gene symbol	Description
TLR4	Toll Like Receptor 4
TMEM232	Transmembrane Protein 232
TMEM26	Transmembrane Protein 26
TNFRSF6B	TNF Receptor Superfamily Member 6b
TP53TG1	TP53 Target 1
TSLP	Thymic Stromal Lymphopoietin
TYRP1	Tyrosinase Related Protein 1
WDR36	WD Repeat Domain 36
ZFAT	Zinc Finger And AT-Hook Domain Containing
ZGPAT	Zinc Finger CCCH-Type And G-Patch Domain Containing
ZNF365	Zinc Finger Protein 365
ZNF652	Zinc Finger Protein 652