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Cohort profile

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Cohort Profile of the HealthNuts study: Population prevalence and environmental/genetic predictors of food allergy

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Abstract

HealthNuts is a single-centre, multi-wave, population-based longitudinal study designed to assess prevalence, determinants, natural history and burden of allergy (particularly food allergy) in the early years of life. It is novel in the use of serial food challenge measures within its population frame to confirm food allergy. The cohort comprises 5276 children initially recruited at age 12 months from council-run immunization sessions across Melbourne, Australia. As well as parent-completed questionnaires and researcher-observed eczema status, all infants underwent skin-prick testing to egg, peanut, sesame and either cow's milk or shellfish, and those with detectable wheals underwent food challenges to determine clinical allergy. In wave 2, conducted at age 4 years, validated questionnaires collected data on asthma, allergic rhinitis (hay fever), eczema and food allergies. Food challenges were repeated in children previously identified as food allergic to determine resolution. In wave 3, all children (irrespective of food allergy status) were invited for clinical assessment at age 6 years, including lung function, physical measurements, skin-prick testing to foods and aeroallergens and food challenges if food sensitized. Biological specimens (blood, cheek swabs) were collected at each wave for ancillary immunological, genetic and epigenetic studies. Applications to access data and/or samples can be submitted to [katrina.allen@mcri.edu.au].

Key Messages

- Food allergy and eczema were evident among one in ten, and one in five, 12-month-old infants in Melbourne, Australia, respectively.
- Potentially modifiable factors associated with decreased risk of food allergy were indicators of possible increased microbial exposure (presence of older siblings and pet dogs), earlier introduction of egg into the infants' diet, and sufficient serum vitamin D levels.
- Non-modifiable risk factors for food allergy included family history of allergy, presence of filaggrin gene loss-of-function mutations and having parents born in East Asia.
- At 1 year of age, higher plasma levels of Th2-related cytokines (IL-4, IL-13, IL12p70) were associated with food sensitization (either with or without associated food allergy), whereas infants with clinical food allergy had lower IL-10 levels.
- Skin-prick tests and serum allergen-specific IgE cut-offs and a peanut component Arah2-specific IgE screening tool were developed to accurately predict challenge-confirmed food allergy in this cohort.

Why was the cohort set up?

In the past few decades, allergic disease has become a major public health concern. Large increases in asthma, eczema and allergic rhinitis have been noted since around the 1960s.[1] Also of concern is new evidence of an increase in food allergy,[2] which may impact on the lifelong health of future generations of children—especially if it represents an early step on the atopic march to other allergic diseases such as asthma. Little is known about the causes and consequences of food allergy at the population level. In response, in 2007 we established HealthNuts, the world's largest single-centre, population-based study of infant and early childhood food allergy. Participants were recruited at age 12 months and followed up at ages 4 and 6 years.

As a multi-wave, population-based longitudinal study, HealthNuts is designed to address a range of questions about the prevalence, determinants, natural history and burden of allergy (particularly food allergy) in the early years of life. Specific areas of enquiry include:

- to determine the population prevalence of sensitization and cross-sensitization to common childhood food allergens, including peanut, and the prevalence of true food allergy, as defined by a positive food challenge;
- to explore risk factors for food allergy in a large population-based sample of infants;
- to determine genetic and/or environmental factors that could identify at-risk infants who might benefit from future novel therapeutic interventions;
- among food-allergic children, to describe precursors (environmental/lifestyle and clinical) of tolerance and persistence of food allergy and the potential of the strongest predictors to underpin new prevention, detection and/or intervention strategies for childhood food allergy;
- to establish determinants, natural history and outcomes (physical, psychosocial, health care utilization) of food allergy, and inter-relationships with other allergic diseases (asthma, hay fever and eczema) at the population level by age 6 years.

Who is in the cohort?

HealthNuts is based in Melbourne, Australia (population 3.9 million in 2008[3]). Infants were recruited between September 2007 and August 2011 when presenting for their routine scheduled vaccination at immunization clinics in greater metropolitan Melbourne and immediate surrounds (within 70 km from the central business district). Immunization coverage at age 1 year is very high in Victoria (92% in 2007) and around half (46%) of Victorian infants are immunized at local-government led sessions, with the remainder immunized by general practitioners (GPs) or state/community health agencies.[4]

The recruitment catchment area encompasses 31 local government areas, 30 of which run regular council-led immunization sessions, and 29 of these agreed to participate in the study. Over 170 immunization session locations were screened for suitability for conducting the HealthNuts study, and participants were recruited from 138 locations. The sampling frame for baseline recruitment has been described in more detail elsewhere.[5] All infants aged between 11 and 15 months (inclusive) attending for their routine 12-month immunizations were eligible for recruitment (mean age 12.7 months). Of 7134 approached, 5276 (74%) participated. Of parents who declined to participate, 94% (1745/1858) completed a questionnaire capturing information on reasons for not participating,

Table 1. Differences between responders and non-responders at baseline

Baseline variable	Participants $N = 5276$	Non-participants $N = 1858$	p-value
Child's sex (% male)	2665/5244 (50.8%)	804/1678 (47.9%)	0.038
History of eczema in the child	1325/4982 (26.6%)	189/1259 (15.0%)	<0.001
Any siblings	2647/5251 (50.4%)	703/1278 (55.0%)	0.003
History of parent or sibling allergic disease	3661/5276 (69.4%)	610/1271 (48.0%)	<0.001
Has child consumed peanuts (% yes)	1527/4654 (32.8%)	738/1239 (59.6%)	<0.001
Socioeconomic status by postcode (SEIFA)[a]	$n = 5261$	$n = 1736$	0.020
1 (most disadvantaged)	19.6%	23.3%	
2	20.5%	19.7%	
3	20.9%	20.9%	
4	19.4%	18.4%	
5 (least disadvantaged)	19.5%	17.7%	

[a]Socioeconomic status was assigned on the basis of home postcode by using Socio-Economic Indexes for Areas (SEIFA) measures, derived from the 2006 Australian census, which assess relative socioeconomic advantage/disadvantage, economic resources (income, assets and expenditure) and educational and occupational characteristics.[30]

allergic history in the infant and family, and postcode. Not surprisingly, infants with a history of eczema, family history of allergy, males (male infants also had more eczema and food allergy), those from an area with higher socioeconomic status, and those who had never eaten peanuts were overrepresented among participants compared with non-participants (Table 1).

How often have they been followed up?

The HealthNuts cohort, recruited at 1 year of age, has now been followed up at ages 4 years and 6 years and planning is under way for further follow-ups. Questionnaire-based data were collected at each wave, with clinical investigation of food allergic and food-sensitized individuals taking place at all three waves (Figure 1). Infants who were egg-allergic at age 1 year underwent additional clinical follow-up at age 2 years to determine whether they had grown out of their egg allergy. At age 4 years, the entire cohort completed a questionnaire and additional clinical assessment was completed in all children undergoing clinical investigation at age 1 year, as well as infants reporting a new reaction to a food. In the third wave (age 6 years), the entire cohort is offered a visit to a study clinic or a home visit for clinical measurements (n = 930 completed to date), and consent is also being sought from participants' parents or guardians for linkage to data registries (see study measures). In addition, consent and ethics approval has been obtained to access blood samples from children's newborn screening cards, which will be used to measure vitamin D levels at birth as well as for genetic and epigenetic analysis. This allows an additional time point (birth) for assessing epigenetic changes over time associated with the onset of and resolution of allergic disease.

Study participation at age 4 years is shown in Figure 2. Overall, contact details were available for 98% of the cohort. Of these, 83% completed a questionnaire at age 4 and 73% of these completed the full 14-page questionnaire. The remaining 27% completed a short version administered by research assistants over the telephone, which captured key information about allergic disease in the child and family members.

Differences between participants followed up at age 4 years and those lost to follow-up are described in Table 2. Children with a history of allergic disease in the first year of life were less likely to be lost to follow-up, as were those with a family history of allergic disease, families where the mother was born in Australia and those with a higher socioeconomic status. Participants completing the short questionnaire were more similar to non-participants than participants completing the full questionnaire.

What has been measured?

Questionnaire-based data, including maternal and infant diet, maternal vitamin and medication use in pregnancy, family history of allergic disease, pet exposure, exposure to tobacco smoke, history of migration and parental country of birth, were collected at each wave (Table 3). Data on outcomes of eczema, asthma and allergic rhinitis were collected using standardized questions from the International Study of Asthma and Allergies in Childhood (ISAAC).[6] This was augmented with nurse-observed eczema measures, aeroallergen skin-prick tests and objective lung function testing starting from ages 1, 4 and 6 years, respectively. Biological specimens were obtained from birth (using newborn screening cards) and each wave for genetic, epigenetic and immunology studies as well as vitamin D testing.

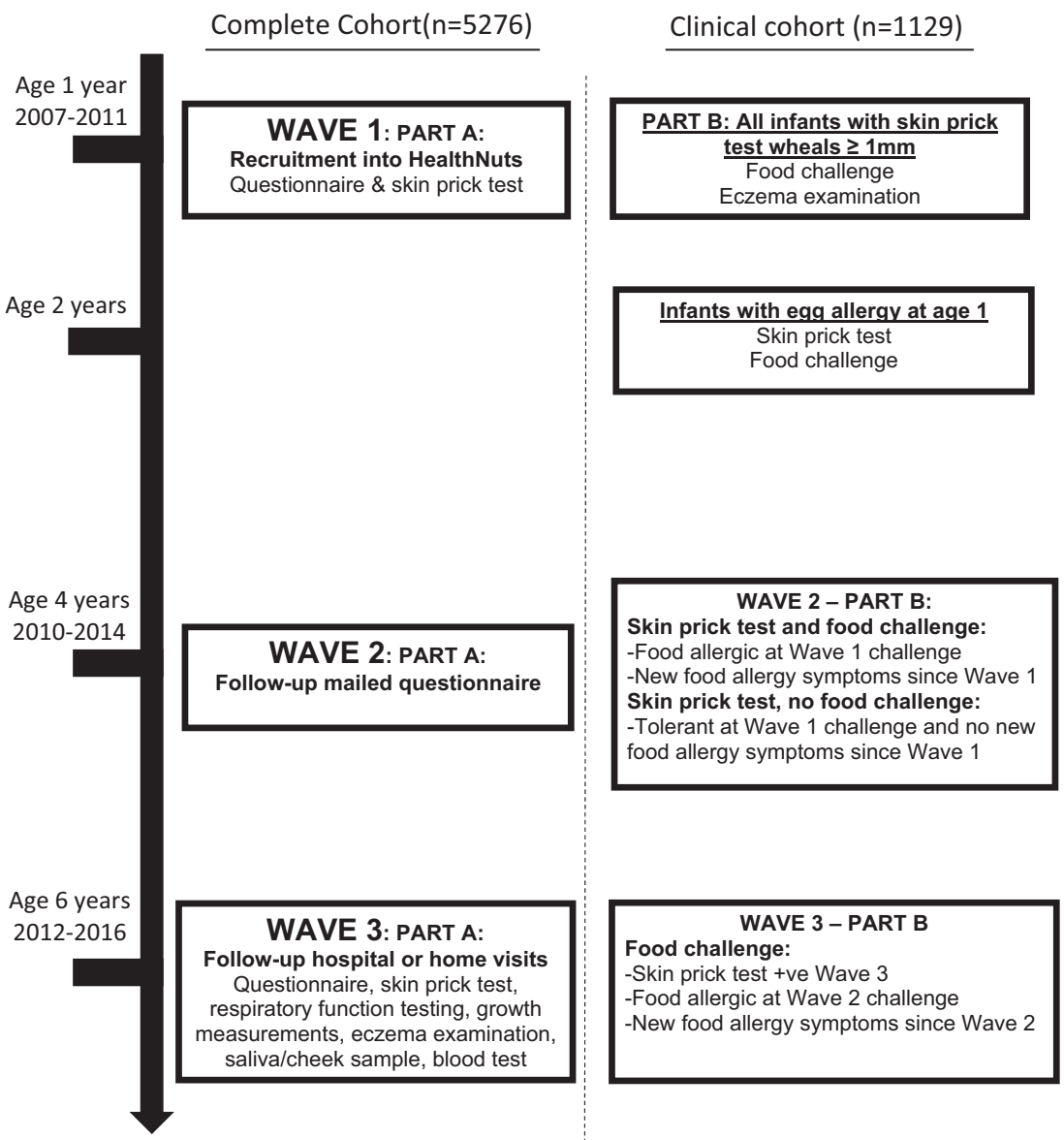

Figure 1. Study protocol for follow-up of the HealthNuts cohort.

For the primary outcome of interest, food allergy, skin-prick tests were performed on the child's back using single-tine metal lancet devices. At 1 year of age, all infants with a detectable wheal to food/s were offered an oral food challenge unless there was a clear history of immediate symptoms of IgE-mediated food allergy following definite exposure to the food within the past 1 month for egg or 2 months for peanut or sesame. Protocols for food challenges were based on standard protocols developed by the Australasian Society for Clinical Immunology and Allergy.[5] Standardized objective discontinuation criteria for challenges were developed as part of the study—the first time that standardized criteria determined a priori have been used in this type of study—and these criteria were shown to be safe and effective for oral food challenges in infants.[7] The objective discontinuation criteria developed as part of HealthNuts were subsequently incorporated into standardized international guidelines for oral food challenges.[8] HealthNuts protocols have been used to establish the prevalence of food allergy internationally including by the South African Food Sensitization and Food Allergy Study (manuscript under review) and Singapore food allergy prevalence study.[9]

Clinical assessments for other outcomes including growth measurements and cardiovascular risk factors (blood pressure and retinal photography) were also conducted as described in Table 3. To measure burden of disease, we also collected information on health care utilization, child and family health-related quality of life,[10,11] and physical and psychosocial functioning including information on problems (emotional, conduct, attention, peer) and prosocial behaviour.[12] Records will be

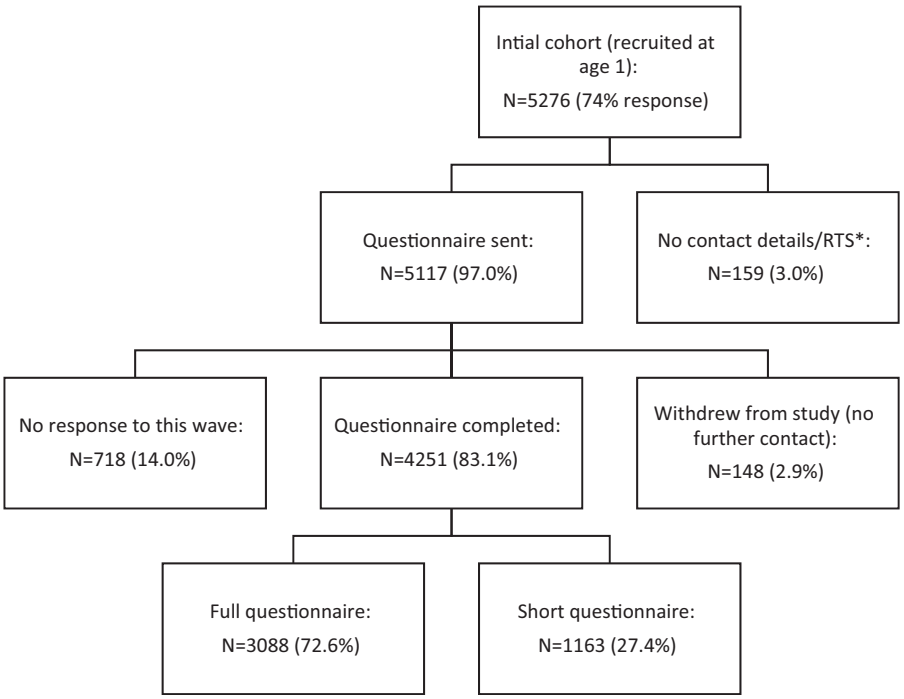

Figure 2. HealthNuts cohort participation and retention up to age 4 years. RTS, 'return to sender', unable to obtain updated contact details.

linked with the Australian Childhood Immunisation Register, Medicare/Pharmaceutical Benefit Scheme to examine adrenaline prescriptions and the School Entrant Health Questionnaire to examine child health at school entry.

What has it found? Key findings and publications

Prevalence and natural history of allergic disease

The HealthNuts study provided the first estimate of challenge-confirmed food allergy prevalence in Australian infants.[13] Of 12-month-old infants, 3% [95% confidence interval (CI) 2.4–3.8] were peanut-allergic, 9% (95% CI 7.8–10.0) were egg-allergic and 0.8% (95% CI 0.5–1.1) were sesame-allergic. By 2 years of age, egg allergy had resolved in 47% of egg-allergic children (95% CI 37–56), with baked egg tolerance at 12 months strongly predicting resolution of egg allergy.[14] Peanut allergy resolved in a smaller percentage of cases, with 22% of peanut-allergic children developing tolerance by age 4 years (manuscript accepted for publication in the Journal of Allergy and Clinical Immunology).

Eczema was also extremely common in this cohort, with 28.0% (95% CI 26.7–29.4) reporting a history of having had eczema in the first year of life, whereas 20.3% (95% CI 19.0–21.5) had eczema at 12 months as observed by the nurse at recruitment.[15] The strongest risk factors for eczema were maternal eczema [odds ratio (OR) 1.7, $p < 0.001$] and asthma (OR 1.4, $p = 0.007$), male sex (OR 1.4, $p < 0.001$), and East Asian ethnicity (OR 1.6, $p < 0.001$).

Risk factors for food allergy

Risk factors for food allergy in this cohort are summarized in Figure 3. Potentially modifiable factors inversely associated with food allergy were increased microbial exposure (presence of older siblings and pet dogs),[16] earlier introduction of egg into the infants' diet[17], and higher serum vitamin D levels.[18] Non-modifiable risk factors for food allergy included eczema, with the risk increasing with earlier onset and more severe eczema,[19] family history of allergy[20] and having parents born in East Asia.[21]. Other exposures hypothesized to play a role in allergic disease, such as maternal consumption of allergenic foods during pregnancy[17] and cesarean section delivery,[16] were not associated with food allergy in this cohort.

In an extension to the work on risk factors for eczema and food allergy, latent class analysis was used to identify phenotypes and risk factors of allergy in infancy (manuscript accepted for publication in the journal of Clinical and Experimental Allergy). This analysis identified five distinct allergy phenotypes: no allergic disease (71%); non-food sensitized eczema (16%); egg allergy (8%); multiple food allergies (predominantly peanut) (3%); and multiple food allergies (predominantly egg) (2%). Shared risk factors for all allergic phenotypes included parental country of birth in Asia, male gender, delayed introduction of egg and

Table 2. Differences in baseline characteristics between responders and those lost to follow up at age 4 years

Baseline variable	Completed 4-year-old questionnaire		Not retained at age 4 years $N = 1025$	p-value
	Full $N = 3088$	Short $N = 1163$		
Child's sex (% male)	51.0	49.4	52.1	0.43
Food allergy at age 1 year	13.0	8.7	6.8	<0.001
Eczema in first year of life	28.0	25.0	24.3	0.033
Parent history allergic disease				
Maternal	47.2	41.6	38.4	<0.001
Paternal	40.1	36.5	33.5	<0.001
Sibling history allergic disease				
No siblings	50.0	46.5	50.8	
Siblings without allergy	30.8	30.5	29.7	
Siblings with allergy	19.2	23.0	19.5	0.047
Socioeconomic status (SEIFA)[a]				
1 (most disadvantaged)	15.8	25.1	27.4	
2	20.1	20.5	19.2	
3	21.8	21.0	18.1	
4	21.2	16.7	17.4	
5 (least disadvantaged)	21.2	16.7	17.9	<0.001
Maternal country of birth				
Australia	78.8	64.5	60.2	
East Asia	7.3	13.8	13.3	
Other	13.9	21.7	26.5	<0.001

[a]Socioeconomic status was assigned on the basis of home postcode by using Socio-Economic Indexes for Areas (SEIFA) measures, derived from the 2006 Australian census, which assess relative socioeconomic advantage/disadvantage, economic resources (income, assets and expenditure) and educational and occupational characteristics.[30]

family history of allergic disease, whereas exposure to pet dogs was protective for all phenotypes. Other factors such as sufficient vitamin D levels and presence of older siblings were protective for only some allergic phenotypes, consistent with potential differential aetiology and natural history of these conditions.

Within the HealthNuts cohort, filaggrin gene (FLG) loss-of-function mutations, which are associated with reduced skin barrier integrity, were significantly more common in infants with food sensitization compared to non-sensitized infants[22]. This supports a potential role for food allergen exposure through a damaged skin barrier in the development of sensitization, as has been suggested by others.[23] Interestingly, the prevalence of FLG null mutations was similar in those with asymptomatic food sensitization and those with symptomatic food allergy. This suggests that other factors might play a role in the development of true food allergy following initial sensitization to foods. One potential factor involved in this second step of converting food sensitization to true food allergy is vitamin D insufficiency. In the HealthNuts cohort, vitamin D insufficiency was far more common among infants with true food allergy compared with sensitized tolerant infants,[18] potentially supporting a role for vitamin D in maintaining tolerance among food-sensitized infants.

Unexpectedly, low serum vitamin D levels were associated with food allergy only among infants with parents born in Australia. Lower vitamin D-binding protein levels increase the biological availability of serum vitamin D, and genetic polymorphisms explain almost 80% of variation in binding protein levels.[24] We therefore explored the hypothesis that polymorphisms affecting binding protein could compensate for adverse effects of low serum vitamin D on food allergy risk. In this cohort, low serum vitamin D levels at 12 months of age were associated with food allergy only among those with higher genotype-predicted vitamin D-binding protein levels, whereas there was no association between serum vitamin D and food allergy in infants with low genotype-predicted vitamin D-binding protein levels (manuscript under review).

Ongoing work is exploring the role of genetic factors, in conjunction with environmental exposures, in the development of allergic disease. As part of this work a genome-wide association study of the HealthNuts cohort has been conducted and results are currently being analysed. This is in addition to a candidate gene approach which has commenced investigation on the role of SPINK5, CD14 and IL13 genes in the development of food allergy.

Diagnosis of food allergy

In view of the stringent methodology (large sample size, systematic screening for sensitization with skin-prick testing and use of food challenges to identify food allergy), the HealthNuts study provides a unique data set to study factors that could improve the diagnosis of food allergy. Skin-prick tests and serum allergen-specific IgE cut-offs were identified which could accurately distinguish between tolerance and allergy at ages 1[25] and 4 years (manuscript accepted for publication in the Journal of Allergy and Clinical Immunology). In addition, recommendations for a more effective screening test for peanut allergy using a two-step process combining peanut specific IgE (by skin-prick testing or serum levels) and peanut component Arah2-specific IgE were developed,[26] both of which reduce the need for performing oral food challenges to diagnose peanut allergy.

Table 3a. Clinical assessments in the HealthNuts cohort

Measures	Birth[a]	Age 1 year (Wave I)	Age 2 years	Age 4 years (Wave II)	Age 6 years (Wave III)
Allergy-related measures					
Skin-prick test using single metal lancet					
– Food allergens (number)[b]		√ (4)	√ (1- egg)	√ (10)	√ (10)
– Aeroallergens (number)				√ (8)	√ (8)
Food challenges		√ (egg, peanut, sesame)	√ (egg)	√ (any food)	√ (any food)
Eczema examination		√	√	√	√ (SCORAD)
Transepidermal water loss (TEWL)		√			
Lung function testing					
– Pre and post bronchodilator spirometry					√
– Expired nitric oxide (FeNO)					√
Asthma/wheezing/coughing				√	√
Allergic rhinitis/hay fever				√	√
Rashes/eczema		√	√	√	√
Other clinical measurements					
Growth measurements					
– Height	√	√		√	√
– Weight	√	√	√	√	√
– Bio-impedance/body fat					√
– Head circumference	√	√			√
– Waist circumference					√
Blood pressure					√
Retinal photography					√
Biosamples					
Blood sample	√	√	√	√	√
– Genetics/epigenetics					
– Vitamin D					
– Immunology					
Saliva sample				√	√
Fecal samples		√ (n = 40)			

SCORAD, Scoring Atopic Dermatitis.

[a]Birth measurements obtained from child's growth and development record. Blood samples obtained from stored newborn screening (Guthrie) cards.

[b]Minimum number of foods tested for all participants. Participants were additionally skin-prick tested to other foods if they reported a history of reaction when attending HealthNuts food challenge clinics.

Immunology of food allergy

Using blood samples collected at 1 year of age, we showed that higher plasma levels of Th2-related cytokines (IL-4, IL-13, IL12p70) were associated with food sensitization (either with or without associated food allergy), whereas those with clinical food allergy had lower IL-10 levels compared with sensitized infants who were tolerant on ingestion of the food. Differences in plasma cytokine levels were evident between egg- and peanut-allergic infants, potentially reflecting different mechanisms for these allergies, which may contribute to explaining the differences in natural history of egg compared with peanut allergy.[27]

Epigenetics of food allergy

In a novel area of investigation, genome-wide DNA methylation profiling was performed on blood mononuclear cells of children with and without clinical food allergy, and a supervised learning approach was used to discover a DNA methylation signature of 96 CpG sites that predict clinical outcomes (manuscript accepted for publication in the journal of allergy and clinical immunology). This methylation signature outperformed allergen-specific IgE for predicting oral food challenge outcomes. Further work within this cohort will attempt to uncover mechanisms involved in explaining gene regulation mediated through environmental exposures and the role of epigenetic mechanisms as an explanation for the rise in food allergy.

Other outcomes

Over the period of study recruitment, Australian guidelines underwent a significant shift to remove recommendations to delay allergenic solids. Although causality is uncertain,

Table 3b. Questionnaire data and data linkage in the HealthNuts cohort

Data	Pregnancy[d]	Age 1 year (Wave I)	Age 2 years	Age 4 years (Wave II)	Age 6 years (Wave III)
Questionnaire measures					
Home address/geocoding		√		√	√
Pregnancy/birth					
Mode of delivery	√				
Gestational age	√				
Maternal vitamins and medication use in pregnancy	√				
Maternal allergenic foods during pregnancy and/or breastfeeding	√				
Family characteristics					
Number of siblings/household size		√		√	√
Family history of allergy		√		√	√
Immigration/language spoken at home		√			
Ethnicity/skin colour					√
Parent education/household income/employment		√		√	
Tobacco smoking, household/maternal/paternal	√	√		√	√
Parent mental health (Kessler psychological distress scale: K6)		√		√	√
Child characteristics					
Sex		√			
Strengths and Difficulties Questionnaire[a]				√	√
Pediatric Quality of Life Inventory (PEDS-QL 4.0)[b]				√	√
Impact of food allergy on quality of life[c]					
Child health					
– Bronchiolitis/wheezing		√			
– Gastrointestinal worm infection				√	√
– Vomiting/diarrhoea		√			
– Colic/reflux		√			
– Antibiotic use	√	√			
Health services utilization					√
Breastfeeding/formula/ age at introduction of foods/diet		√		√	√
Sleep duration				√	√
Physical activity					√
Environmental exposures					
– Child care		√		√	√
– Pets		√		√	√
– Sun exposure				√	√
Data linkage					
– Australian Childhood Immunisation Register (ACIR)					√
– Medicare/Pharmaceutical Benefits Scheme (PBS)					√
– School Entrant Health Questionnaire data					√
– Victorian perinatal database					√

[a]Widely-used 25-item child mental health questionnaire probing problems (emotional, conduct, attention, peer) and prosocial behaviour.[12]

[b]Peds-QL™4.0: 23-item parent-proxy yields Total, Physical and Psychosocial scores; widely used to measure child health-related quality of life with excellent psychometric validity. Family Impact Module: 8 items ($\alpha = 0.90$) summarizing parent perception of how the child's health impacts on family activities and relationships.[10]

[c]Parent-proxy validated allergy child health perception measure developed by the Europrevall study to assess the impact of food-allergy specific burden of disease.[11]

[d]Parent reported at recruitment.

we showed that this change in guidelines was followed by a reduction in the proportion of parents delaying introduction of solids, egg and peanut to their infant, with better uptake of infant feeding guidelines among families with higher socioeconomic status and absence of family history of allergies.[28]

At age 1 year, approximately 14% of the population had a history of gastro-oesophageal reflux (GOR) symptoms leading to medical consultations, with high use of anti-reflux medication despite the absence of evidence that it is appropriate or effective for uncomplicated GOR. Risk factors included prematurity and family history of atopy.[29]

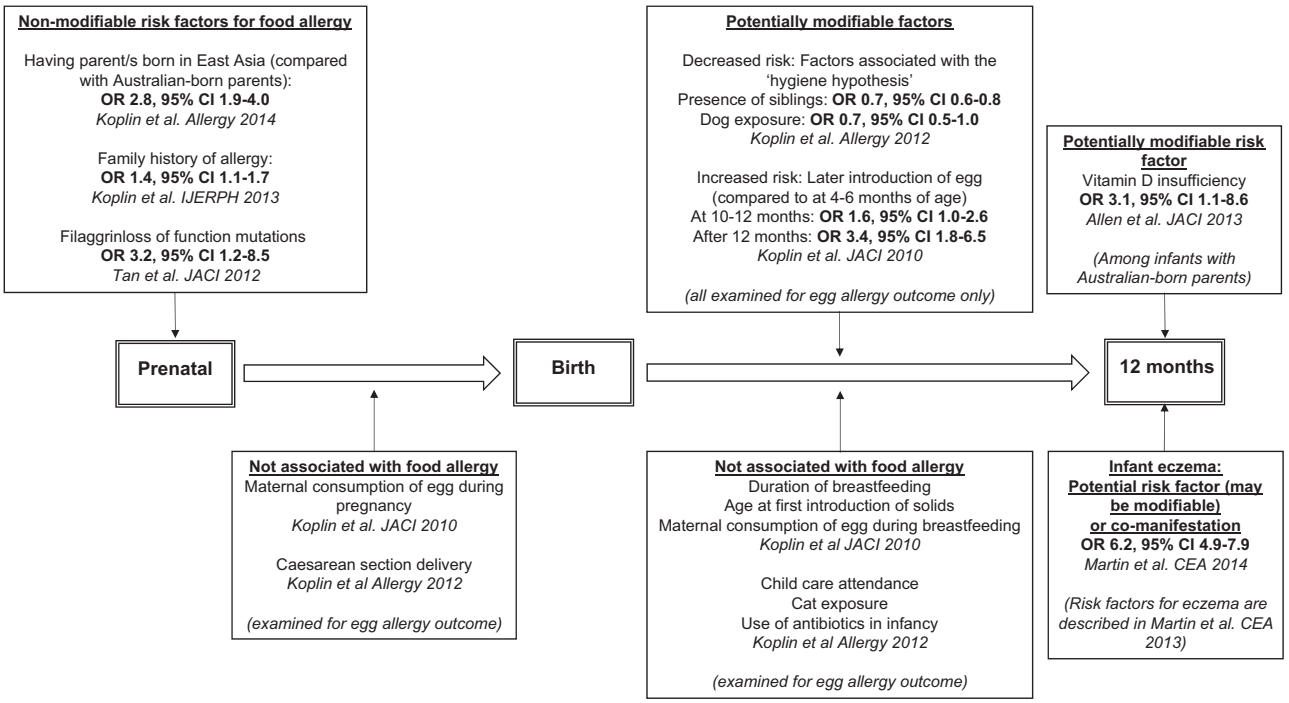

Figure 3. Factors examined for association with infantile food allergy in the HealthNuts cohort. IJERPH, International Journal of Environmental Research and Public Health; JACI, Journal of Allergy and Clinical Immunology; CEA, Clinical and Experimental Allergy.

At 1 year of age, 5% of infants in HealthNuts had above-normal body mass index (BMI), using the World health Organization (WHO) Child Growth standard classification. Longer duration of full breastfeeding and introduction of solids between 4 and 6 months of age were associated with lower odds of above-normal BMI after adjustment for potential confounders.

Environmental and genetic factors associated with vitamin D insufficiency at age 12 months were also explored. Vitamin D insufficiency was more common in infants homozygous (AA) for single nucleotide polymorphism rs4588 in the vitamin D-binding protein (GC) gene, and was less common in formula-fed infants.[29]

What are the main strengths and weaknesses?

The major strengths of HealthNuts are the population-based sample, collection of information on non-responders to evaluate participation bias (allowing prevalence estimates for food allergy to be back-weighted to better reflect population estimates), standardized measurement of food allergy outcomes using the gold standard of oral food challenges in conjunction with skin-prick test screening, and the large sample size. The sample size of 5000 was prospectively calculated to provide sufficient power to detect risk factors present in at least 10% of the population, given a prevalence of food allergy of 5% to 10%.

HealthNuts recruited infants at 12 months of age, which has both advantages and disadvantages compared with other study designs. The less intensive nature of the study, with questionnaires designed to be completed during the compulsory 15-min waiting period after immunization and skin-prick testing taking place at recruitment, made it possible to recruit a large number of participants at a relatively low cost, and to achieve high response rates. Since there were multiple potential hypotheses of interest regarding risk factors for food allergy at the time of starting the study, and little existing evidence to guide research into specific exposures, data collection was on a broad range of potential risk factors. Detail in exploring some of the interesting associations was therefore limited; however, linkage to databases and collection of additional information at ages 4 and 6 years (for variables such as grandparental ethnicity, which do not change over time) provide a greater depth of data. Although a birth cohort allows for prospective collection of samples and data, recruitment at birth into an allergy study might lead to changes in behaviour if parents seek to become more informed about allergy prevention guidelines.

As HealthNuts was initially designed as a cross-sectional study, parents were initially unaware that they would be asked to participate in a follow-up. This posed challenges for following up participants, and possibly reduced retention. Collection of additional contact details at baseline (e.g. for grandparents or other family members

living at a different address) might have been helpful for participant tracing.

Can I get hold of the data? Where can I find out more?

Applications to explore research questions using existing data or biological samples can be submitted to the Principal Investigator, Katrina Allen at [katrina.allen@mcri.edu.au]. The HealthNuts study is also part of the newly established LifeCourse initiative, based at the Murdoch Childrens Research Institute, which is bringing together resources and data to address new research questions on development and communicable and non-communicable diseases [http://mcclr.melbournechildrens.com/study-index/healthnuts/].

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Conflict of interest: M.T. is a member of the Medical Advisory Board (Oceania) for Nestle Nutrition Institute, a member of the Medical Advisory Board (Australia New Zealand) for Danone Nutricia and a member of the Scientific Advisory Board for Immunology Allergy (Global) for Danone Nutricia; ahas received lecture fees from Danone and Nestle Nutrition Institute; and has received travel fees from APAPARI. K.A has received speaker's honoraria from Abbott, Danone, Nestle and Alphapharm. The rest of the authors declare that they have no relevant conflicts of interest.

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