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Cohort Profile

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Cohort Profile: Melbourne Atopy Cohort study (MACS)

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Why was the cohort set up?

The Melbourne Atopy Cohort Study (MACS) is a longitudinal study of a high-risk birth cohort, and their families, that focuses on the natural history, causes and consequences of allergic diseases (eczema, food allergy, asthma and allergic rhinitis). There is evidence that the prevalences of asthma, eczema and allergic rhinitis have risen rapidly since the 1960s[1] and, although the evidence is less robust, there also appears to have been a later increase in the prevalence of food allergy.[2] Australia has one of the highest rates of these diseases in the world.[3,4] The rapid rise in prevalence suggests that shifts in environmental exposures are important in the induction of these diseases, as genetic influences alone cannot account for such swift changes.

For these reasons we established the MACS in 1990, by recruiting 620 unborn infants (probands) and their family members (1234 parents and 617 siblings). The MACS was originally designed to trial the effect of three infant formulae on the incidence of allergic disease. Before birth, infants were randomized to receive one of three formulae (cows' milk, soy and a partially hydrolysed whey formula) at cessation or partial cessation of breastfeeding. Further details of this clinical trial are published elsewhere.[5] Although the MACS was commenced as a clinical trial, the data have

Table 1. Baseline demographic factors

	Maternal (n = 620)	Paternal (n = 617)	
Mean age (SD)	31.2 (4.4)	33.4 (5.3)	
Completed tertiary education (%)	364 (58.7%)	377 (61.1%)	
Birthplace (% Australia/NZ)	538 (86.8%)	510 (82.5%)	
Marital status (% married)	564 (91.0%)	563 (91.3%)	
Residence (% owner occupied)	494 (79.7%)	493/613 (80.4%)	
Smoking			
(% current)	39 (6.3%)	112/615 (18.2%)	
(% never)	461 (74.4%)	409/615 (66.5%)	
Social Security (% receiving)	22 (3.6%)	19/613 (3.1%)	
Baseline family history of atopic disease			
	Maternal (n = 619)	**Paternal (n = 615)**	**Any older sibling (n = 371)**
Food allergies			
Milk (%)	123 (19.9%)	61 (9.9%)	163/370 (44.1%)
Egg (%)	53 (8.6%)	30 (4.9%)	104/370 (28.1%)
Other (%)	190 (30.7%)	94 (15.3%)	186/370 (50.3%)
Any (%)	239 (38.6%)	131 (21.3%)	231/370 (62.5%)
Eczema (%)	241 (38.9%)	126 (20.5%)	238 (64.2%)
Asthma (%)	268 (43.3%)	158 (25.7%)	219 (59.0%)
Hay fever (%)	375 (60.6%)	284 (46.2%)	135/369 (36.6%)
Any atopic disease (%)	513 (82.7%)	375 (61.0%)	337 (90.8%)

been subsequently used to address a wide range of questions relating to the natural history of allergic disease and risk factors for these conditions. Specific areas of focus are:

- oral and environmental risk factors for allergic disease and impaired lung function;
- use of early life biomarkers, particularly skin prick testing, to identify children at the greatest risk of developing allergic disease;
- and examination of the natural history and interrelationships between the manifestations of allergic diseases, including wheeze sub-types.

Who is in the cohort?

The MACS is a single-centre study of children with a family history of allergic disease. The MACS is based in Melbourne, Australia (population 4.35 million in 2013).[6] Expectant mothers were approached during antenatal care visits at the former Mercy Hospital for Women (East Melbourne) and other private consulting suites. Children with a parent or older sibling with a history of allergic disease (asthma, eczema, allergic rhinitis or severe food allergy) were eligible to be enrolled. Infants were born between 1990 and 1994. Parents provided informed consent, and the Mercy Hospital Human Ethics Committee granted ethical approval. The parents of the MACS were highly educated, predominantly Australian born, and had a high rate of allergic disease with the most common condition being hay fever (Table 1)

How often have they been followed up?

The baseline study included 620 probands and 1851 family members. The MACS probands were followed up 18 times in the first 2 years of life (every 4 weeks until 64 weeks, then at 18 months and 2 years) by use of a telephone survey administered by an allergy trained nurse (Figure 1). Clinical visits for skin prick testing (SPT) were performed at 6, 12 and 24 months. An annual telephone survey was performed from 3 to 7 years of age. At 12 years of age, children were invited to attend a clinical examination and complete a survey. At 18 years, probands, parents and siblings were invited to undergo a detailed clinical examination. The 25-year follow-up of the MACS families has just commenced (2015); this will include surveys and clinical examination of probands and siblings, and surveys of the parents.

Approximately 93% of MACS participants were still under observation at 2 years of age (Figure 1). The rate of follow-up at 6 years was 78%, and this declined to 58% at 12 years. A major effort was undertaken to trace lost MACS participants for the 18-year follow-up, using telephone directory and electoral roll searches and assisted Medicare Australia mail-outs, which resulted in a follow-up of 68% (423/620 plus 512 siblings and 691 parents).

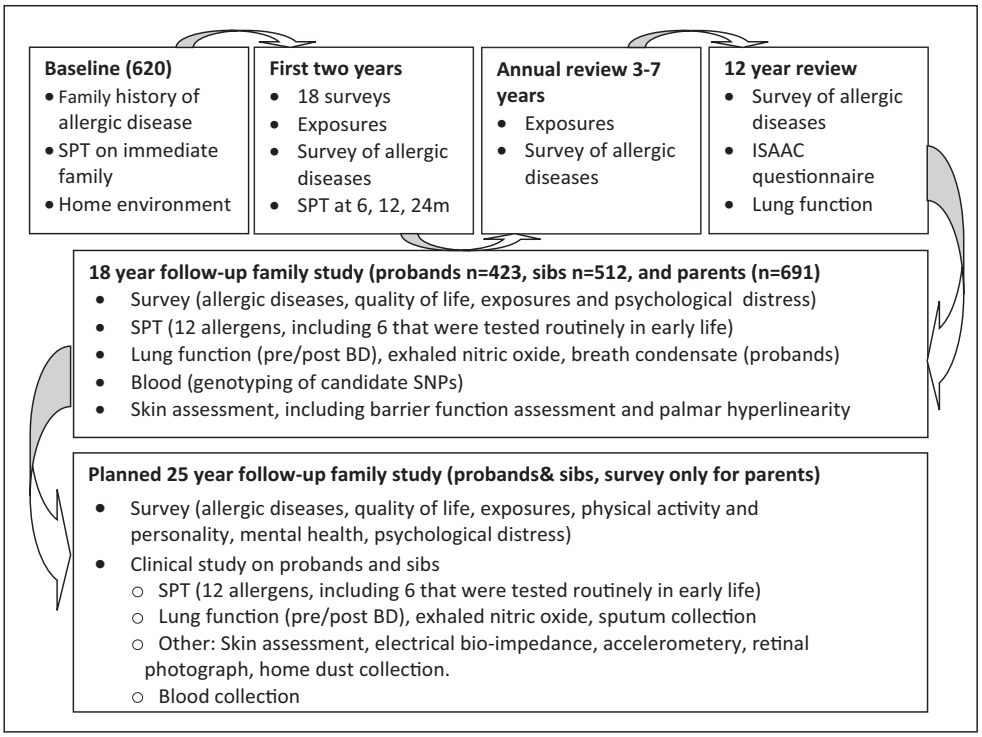

Figure 1. Study protocol for follow up of the MACS. ISAAC, International Study of Asthma and Allergy in the Child; SPT, skin prick test; m, months; BD, bronchodilator; SNPS, single nucleotide polymporphisms.

There has been minimal evidence of selective participation attrition within the MACS. Children who were lost to follow-up were more likely to have had younger and less educated parents than those who were retained in the cohort (Table 2). Children who were lost to follow-up were also more likely to have a mother with self-reported hay fever, but only for those lost by 2 years of age. At 2 and 18 years, children of parents who smoked were more likely to be lost (Table 2). Children of parents who were renting their home, and whose father's occupation was associated with a lower socioeconomic status, were more likely to be lost. There were no apparent differences between children lost to follow-up and those studied in terms of gender, family history of other allergic diseases (other than maternal hay fever), early signs of atopy or eczema in the child, or a range of residential characteristics including pet ownership.

What has been measured?

At baseline, data were collected on family history of allergic disease, pet keeping, tobacco smoking, heating and cooking facilities in the family home (Table 3a). From birth to 2 years, 18 telephone surveys were used to collect data on outcomes of eczema and rash, wheeze, reactions to foods and detailed information on infant diet. Data on medication use, including indication for use, were also collected at these times. From 3 years onwards, parental reports of asthma, allergic rhinitis and food allergy were collected. Skin prick tests (SPT) were performed at 6, 12 and 24 months, and then at 12 years using a panel of three food and three aero allergens (Table 3b). At 12 and 18 years, the standardized questions from the International Study of Asthma and Allergies in Childhood (ISAAC)[7] on allergic disease symptoms were also administered. At 18 years, the SPT panel was expanded to five food and six aero allergens. Lung function testing was performed at both 12 and 18 years, and this included measurements following inhaled bronchodilator at 18 years. Anthropomorphic measurements were collected at various ages. Questions about quality of life and psychological distress were completed at the 18-year follow up.

First-degree family members (parents and older siblings) were also offered a SPT during the participants' first 2 years of life (including 511 mothers and 399 fathers), as well as at the 18-year follow-up. Siblings and parents were also recruited into the 18-year follow-up, and the same outcome measures as for probands were collected at this time. In addition, we have requested permission to access health care utilization data for participants and their families: hospitalizations (Victorian Admitted Episodes Database), Emergency Department presentations

Table 2. Associations (ORs) between baseline variables and loss to follow-up at each follow-up period

Baseline variable[a]	2 years (45/620)	6 or 7 years (125/620)	12 years (248/620)	18 years (188/620)
Maternal hay fever (375)	**2.11 (1.04–4.25)**	1.10 (0.73–1.65)	1.21 (0.87–1.68)	1.04 (0.73–1.47)
Fathers occupation class[b]	0.92 (0.79–1.07)	0.96 (0.87–1.05)	0.94 (0.87–1.02)	**0.84 (0.77–0.91)**
Home owner (vs renting)	**0.21 (0.11–0.40)**	**0.25 (0.16–0.39)**	**0.40 (0.26–0.60)**	**0.45 (0.30–0.69)**
Parent age				
Maternal (per year)	**0.88 (0.82–0.94)**	**0.89 (0.85–0.94)**	**0.91 (0.88–0.95)**	**0.93 (0.89–0.97)**
Paternal (per year)	**0.91 (0.85–0.97)**	**0.91 (0.87–0.95)**	**0.92 (0.89–0.95)**	**0.96 (0.92–0.99)**
Parental tertiary degree				
Maternal (364)	**0.32 (0.17–0.62)**	**0.53 (0.36–0.79)**	**0.69 (0.50–0.95)**	**0.53 (0.37–0.75)**
Paternal (377/617)	0.61 (0.33–1.14)	0.75 (0.51–1.12)	0.96 (0.69–1.34)	**0.53 (0.37–0.75)**
Parental smoking				
Maternal (47)	**2.97 (1.30–6.82)**	1.08 (0.52–2.23)	1.12 (0.61–2.05)	**2.16 (1.18–3.93)**
Paternal (119/615)	**2.15 (1.10–4.20)**	1.40 (0.87–2.25)	1.46 (0.97–2.18)	**2.36 (1.56–3.57)**

[a]Potential predictors of loss considered, but not listed because they were not associated with risk of loss to follow-up at any point, were: infant gender, maternal, paternal and sibling history of asthma, hay-fever, eczema, food allergy, presence of older siblings, early signs of allergic disease in the child (sensitization at 6, 12 and 24 months, and early life eczema), residential characteristics (cooking and heating facilities, pet ownership).

[b]Father's occupational class categorized using the Australian National University (ANU)-3 system, which ranges from 0 to 100, with higher values indicating higher socioeconomic status associated with the occupation title. ORs rescaled to represent a 10-unit increase in ANU-3 score.

(Victorian Emergency Minimum Dataset), medical consultations (Medical Benefits Scheme) and subsidized pharmaceuticals (Pharmaceutical Benefits Scheme).

A range of biospecimens have been collected from participants. Cord ($n = 305$) or heel/scalp prick blood ($n = 75$) was collected from probands. Colostrum ($n = 194$) and breast milk ($n = 118$) samples were collected from a subset of mothers, and we have measured the long-chain polyunsaturated fatty acids (n-3 and n-6). We are currently measuring human breast-milk oligosaccharides and microbiome in these samples. Blood from the 18-year follow-up was collected and has been stored as whole blood, serum, plasma, lymphocytes, non-lymphocytes and dried blood spots. All blood and milk samples are stored at -80°C. Participants who were unwilling to provide blood at the 18-year follow-up donated a saliva sample for DNA extraction. To date, probands (and siblings) have been genotyped for 110 single nucleotide polymorphisms in various candidate genes. We have obtained ethics approval and consent to access dried blood spots from participants and their siblings that were collected as newborn screening (Guthrie) cards. These samples will be used to quantify neonatal vitamin D levels and to relate these to the incidence of allergic disease.

What has it found? Key findings and publications

Effect of infant formula on allergic disease outcomes

The MACS is the second largest randomized controlled trial (RCT) investigating the role of infant formula in the incidence of allergic disease.[5] In the intention-to treat-analysis of this study of high-risk infants, there was no evidence that either soy [odds ratio, 1.26; 95% confidence interval (CI), 0.84-1.88) or a partially hydrolysed whey formula (1.21; 95% CI, 0.81-1.80) reduced the incidence of allergic manifestations up to 2 years of age. There was also no evidence of reduced prevalence of allergic sensitization in the first 2 years of life, nor asthma or allergic rhinitis up to 7 years of age. These results are in conflict with a number of current international allergic disease prevention guidelines, which still recommend use of a partially hydrolysed formula for allergic disease prevention. We are currently examining the long-term effects (12- and 18-year outcomes) of these infant formulae, including lung function development (manuscript in preparation). This is the first study to follow up the use of these formulas over this time period.

Atopic march hypothesis

Data from the MACS cohort have been pivotal in addressing the 'atopic march' hypothesis. We were one of the first groups to demonstrate that eczema within the first 6 months is associated with an increased risk of new food and aero allergen sensitization,[8] and we have additionally observed that eczema, particularly if early-onset[9] or associated with sensitization,[10] is strongly associated with an increased risk of asthma and allergic rhinitis. This has strengthened the 'atopic march' hypothesis,[11] where eczema may lead to subsequent allergic airways disease, and has contributed to the knowledge basis to justify skin barrier intervention trials.[12–14].

Table 3a. Questionnaire data and data linkage in the MACS

Questionnaire measures	Pregnancy	0-2 years (collected 18 times)	3-7 years	12 years	18 years	Planned 25 years
Home address/geocoding		√		√	√	√
Pregnancy/birth						
Mode of delivery	√					
Gestational age	√					
Family characteristics						
Number of siblings/ household size		√	√	√	√	√
Family history of allergy		√			√	√
Parent education /employment	√				√	√
Tobacco smoking: household/maternal/paternal	√			√	√	√
Child characteristics						
Sex		√				
Child health						
• Bronchiolitis/wheezing /asthma		√	√	√	√	√
• Eczema/rash		√	√	√	√	√
• Allergic rhinitis			√	√	√	√
• Vomiting/diarrhoea		√	√	√	√	√
• Colic/reflux		√				
• Antibiotic use		√				
Psychological distress / quality of life						
Asthma-related attitudes and health beliefs					√	√
Health services utilization					√	
Breastfeeding / formula / age at introduction of foods / diet		√				
Physical activity					√	√
Environmental exposures						
• Childcare		√		√	√	√
• Pets		√		√	√	√
Data linkage						
• Medicare/Pharmaceutical Benefits Scheme (PBS)					√	
• hospitalization (VAED) and emergency department (VEMD) visit data					√	
• Victorian perinatal database					√	

Table 3b. Clinical assessments and biological samples collected in the MACS

Pregnancy	0-2 years	3-7 years	12 years	18 years	Planned 25 years
Allergy-related measures					
Skin prick test	6, 12 and 24 months			As per 0-2 years plus	
• Food allergens (number)	3 (milk, egg, peanut)		3	5 (cashew and shrimp)	5
• Aero allergens (number)	3 (dust mite, rye grass)		3	6 (mixed grass pollen, *Alternaria, Cladisporium, Homodendrum, Penicillium*	6
Eczema examination				√	√
Trans-epidermal water loss (TEWL)				√	
Lung function testing					
• Pre-bronchodilator spirometry			√	√	√
• Post-bronchodilator spirometry				√	√
• Fraction of expired nitric oxide (FeNO)				√	√
Rashes/eczema	√			√	√
Other clinical measurements					
Growth measurements					
• Length/height	√[a]			√	√
• Weight	√[a]			√	√
• Bio-impedance/body fat					√
• Head circumference	√[a]				√
• Waist/hip circumference				√	√
Blood pressure					√
Retinal photography					√
Biosamples					
Colostrum and breast milk					
• Long-chain fatty acids	√				
• Oligosaccharides	√				
• Microbiome	√				
Cord or heel-prick blood	√				
Blood sample					
• Genetics				√	√
• Vitamin D	√[b]			√	√
Saliva sample				√	√

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

Early life risk factors for allergic disease in childhood

Early life exposure to paracetamol has been postulated as a potential cause for asthma through depletion of airway antioxidants. Epidemiological data have shown that exposure to paracetamol in infancy is associated with increased risk of childhood asthma,[15] leading some to call for the discontinuation of its use.[16] Using MACS data, we demonstrated that, whereas increasing frequency of exposure was associated with increased prevalence of asthma in childhood, the association was most likely due to confounding by indication.[17] Paracetamol is commonly used to treat respiratory tract infections, which are in turn a known risk factor for asthma. Adjustment for frequency of respiratory tract infections reduced the strength of this association, and there was no evidence of an association between paracetamol administered for non-respiratory tract indications and childhood asthma. There remains debate on this topic, with many still calling for randomized trials to resolve this issue.[18]

The nature of associations between environmental exposures in early life and allergic disease outcomes remains controversial, and we have used data from MACS to address some of these issues. Historically, early life pet keeping was discouraged, as it was thought to increase risk of allergic disease. However, in MACS, keeping a cat (28.3%) or dog (31.2%) at the time of birth was both common and not associated with allergic disease risk in the

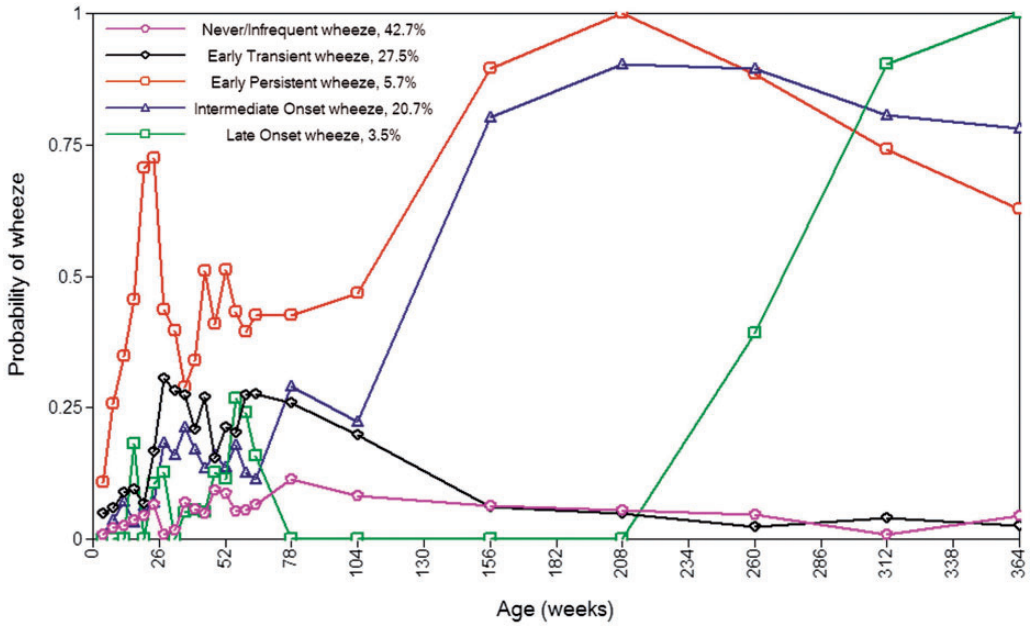

Figure 2. Probability of wheeze at each time point for each wheeze class identified using latent class analysis.

Reproduced with permission from: Lodge CJ, *et al.* Early-life risk factors for childhood wheeze phenotypes in a high-risk birth cohort. *J Pediatr* 2014;**164**:289-94 e1-2.

child. In the subset of children whose father was not sensitized, pet keeping was actually protective against hay fever and allergic sensitization.[19] These and other similar findings have resulted in a change in various guideline recommendations, that no longer include comments concerning pet avoidance for allergic disease prevention.[20]

Although seasonal patterns of allergic disease symptoms are well known, there is also some evidence that season of birth is important in determining the risk of children developing these conditions. There are several hypotheses to possibly explain these seasonal effects, including pollen exposure. We have used data from MACS to demonstrate that cumulative pollen exposure up to 3 months of age was associated with increased risk of both aero allergen sensitization and hay fever at 6/7 years of age.[21]

Wheeze phenotypes: identification, risk factors and prognosis

Asthma is a heterogeneous syndrome with common respiratory manifestations. Attempts have been made to categorize asthma into phenotypes to identify specific aetiological risk factors, the natural histories and response to treatments. In MACS, we classified the cohort into phenotypes utilizing 23 observations of presence or absence of wheeze over the first 7 years of life. Latent class analysis identified five distinct phenotypes of wheeze (Figure 2), similar to those identified previously in other cohorts.[22] These five distinct phenotypes appeared consistent with previous mechanistic hypotheses of childhood wheeze/asthma.[23] The classes were never/infrequent wheeze, the early respiratory infection-related phenotype of early transient wheeze, early persistent wheeze consistent with the 'two-hit' hypothesis,[24] a relatively novel atopic phenotype of intermediate-onset wheeze and the smoking-related phenotype of late-onset wheeze.[23] Furthermore, we investigated respiratory outcomes at 12 and 18 years of age for each phenotype, finding that persistent wheeze phenotypes in childhood (early persistent, intermediate-onset and late-onset) were associated with reduced growth in pre-bronchodilator forced expiratory volume in 1 s (FEV_1) over adolescence.[25] The intermediate-onset wheezers were most affected, and showed irreversible airflow limitation by 18 years. These studies have helped to elucidate causes and refine definitions of the various 'asthmas' in early life, and may help lead to specific and targeted intervention strategies.

Early life biomarkers

We have collected a range of biological samples within the MACS, and have explored a number of potential biomarkers with the aim of identifying children at very-high risk of developing allergic disease, so that interventions can be put in place to prevent this in future studies. As approximately 70% of the infants born in Melbourne have a family history of allergic disease,[26] family history is an indiscriminate marker of allergy risk. However, we have observed that house dust mite sensitization at the age of 1 and 2 years was associated with increased risk of asthma at age 12 years

(especially in eczematous or wheezy infants).[27] Low levels of cord blood interferon gamma were associated with increased risk of both eczema and allergic sensitization.[28] Unexpectedly, high levels of the long-chain polyunsaturated fatty acid n-3 in colostrum were associated with an increased risk of sensitization to food allergens at 6 months and aero allergens at 24 months,[29] but not allergic disease outcomes.[30] These remain ongoing areas of research.

What are the main strengths and weaknesses?

The major strengths of the MACS are the detailed and prospectively collected data on a range of risk factors and exposures in early life (18 surveys by age 2 years), and long follow-up period with high response rate into adult life. Repeated skin prick test data, including very early in life, allow for the evaluation of the natural history and associations between early life sensitization and subsequent outcomes. The prolonged and repeated follow-up allow for the evaluation of the changing expression of allergic disease with age, and associations between the different manifestations. This frequent prospective data collection in early life minimizes potential for recall bias.

This study also has a number of limitations. Although the original sample is substantial, the study lacks power for assessing associations between rare exposures and outcomes. As with all longitudinal studies, participants have been lost over time, and it is difficult to exclude the possibility of selective attrition. The study sample was restricted to those with a family history of allergic disease, which has increased the incidence of disease within the cohort, thus increasing the relative power to detect associations within the study. Infants with a family history of allergic disease are an important population, as guidelines on prevention are often targeted to this group. However, the selection of this sample also means that the results cannot be directly applied to the general population.

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

We welcome applications to utilize the existing MACS datasets or biological samples, and such applications can be submitted to the Principal Investigator, Professor Shyamali Dharmage, at [s.dharmage@unimelb.edu.au].

MACS profile in a nutshell

- MACS is a prospective family study investigating the influence of early life exposures on the natural history, causes and consequences of allergic diseases across the life course in children with a family history of allergic disease.
- A total of 620 children with a family history of allergic diseases were recruited from pregnancies where mothers resided in Melbourne, Australia, and were born between 24 March 1990 and 11 January 1994. Data on first-degree family members have also been collected.
- Follow-up is ongoing, and has included 26 questionnaire surveys (baseline–18 years of age) and five clinical assessment visits (6 months–18 years of age) to date, with a 25-year follow-up about to commence. Out of the original 620 children, 435 children plus their siblings (n = 423) and parents (n = 691) remain eligible for future follow-up.
- The dataset comprises a wide range of phenotypic and environmental measures, biological samples, genetic information and linkage to health and administrative records.
- The MACS investigators support requests to access these data, and requests can be submitted to [s.dharmage@unimelb.edu.au].

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Conflict of interest: K.J.A. is a board member for the Ilhan Food Allergy Foundation and has received lecture fees from Pfizer, Nutricia, Annual Women's Update and Abbott. M.A. holds investigator-initiated grants from Pfizer and Boehringer-Ingelheim for unrelated research, and has received assistance with conference attendance from Boehringer-Ingelheim and Sanofi. A.J.L. received a PhD scholarship from Dairy Australia (2003-07). The rest of the authors declare that they have no relevant conflicts of interest.

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