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Cohort Profile

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Cohort Profile: The Barwon Infant Study

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Summary

The modern environment is associated with an increasing burden of non-communicable diseases (NCDs). Mounting evidence implicates environmental exposures, experienced early in life (including *in utero*), in the aetiology of many NCDs, though the cellular/molecular mechanism(s) underlying this elevated risk across the life course remain unclear. Epigenetic variation has emerged as a candidate mediator of such effects. The Barwon Infant Study (BIS) is a population-derived birth cohort study ($n = 1074$ infants) with antenatal recruitment, conducted in the south-east of Australia (Victoria). BIS has been designed to facilitate a detailed mechanistic investigation of development within an epidemiological framework. The broad objectives are to investigate the role of specific environmental factors, gut microbiota and epigenetic variation in early-life development, and subsequent immune, allergic, cardiovascular, respiratory and neurodevelopmental outcomes. Participants have been reviewed at birth and at 1, 6, 9 and 12 months, with 2- and 4-year reviews under way. Biological samples and measures include: maternal blood, faeces and urine during pregnancy; infant urine, faeces and blood at regular intervals during the first 4 years; lung function at 1 month and 4 years; cardiovascular assessment at 1 month and 4 years; skin-prick allergy testing and food challenge at 1 year; and neurodevelopmental assessment at 9 months, 2 and 4 years. Data access enquiries can be made at [www.barwoninfantstudy.org.au] or via [peter.vuillermin@deakin.edu.au].

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Key Messages

- BIS has been designed to investigate during early life: (i) the relationship between the maternal and infant gut microbiome, epigenetic profile and immune development; (ii) the determinants of lung development and function; (iii) the initiation and potentiation of atherosclerosis and cardiovascular disease risk; and (iv) factors contributing to neurodevelopmental outcomes.
- A series of nested case-cohort studies is being conducted in order to address research questions relating to biological mechanisms.
- Integrated cohort-wide genomic and epigenomic analysis will investigate molecular mediators of risk for a wide range of NCDs.
- Maternal folate levels during pregnancy may influence the epigenetic profile of the developing infant and were higher in BIS than reported in previous population-derived pregnancy cohorts.

Why was the study set up?

To reduce the burden of NCDs in the modern environment, we need a detailed understanding of the mechanisms by which specific early-life environmental exposures predispose to subsequent health outcomes. In the current era of 'omics' technology,[1] the Barwon Infant Study (BIS) has been designed to facilitate laboratory investigation of development and disease in the context of a population-derived antenatally recruited cohort with detailed environmental data and extensive, longitudinally assembled biological specimens. BIS involves an overarching investigation of potential mediators of environmental risk such as the human epigenome (BIS Epigenome) and microbiome (BIS Microbiome). With respect to disease-related phenotype measurement, the aims are characterized with reference to biological systems: BIS Immune, BIS Respiratory, BIS Cardiovascular and BIS Neurodevelopment.

BIS Epigenome

The objective of BIS Epigenome is to investigate the interplay between genetic, environmental and epigenetic factors, both to define the level of variation in the early-life epigenome in response to specific exposures, and also to identify epigenetic variation associated with healthy development and disease. Considerable evidence exists in support of the Developmental Origins of Health and Disease (DOHaD) hypothesis that implicates early-life environmental exposures as altering non-communicable disease (NCD) risk; and epigenetic variation has emerged as candidate mediator of such long-term 'programming' effects.[2–6] However, establishing a role for early-life epigenetic variability as a mediator of NCDs is challenging, particularly as epigenetic marks are subject to genetic, temporal and spatial (tissue-specific) variability. As such, the importance of epigenetics cannot be reliably inferred retrospectively, nor from non-target tissue.[7] In this context, BIS Epigenome has been designed to address a wide range of hypotheses along a proposed causal pathway (Figure 1), by maximizing exposure-related data, diversity of collected biospecimens, and phenotypic data (including clinical parameters) at multiple time points commencing prior to birth. Our initial aim is to test the hypothesis that a subset of methylation-sensitive genes (MSGs), regulating naïve

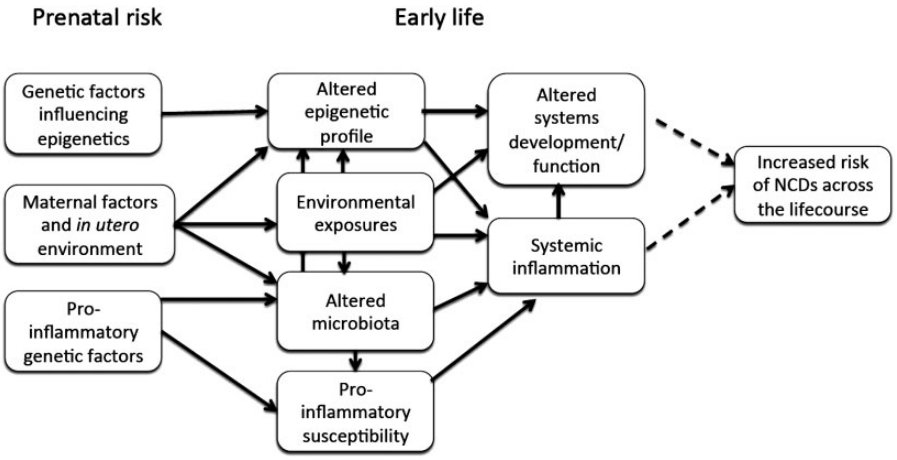

Figure 1. The overall mediation model under investigation in BIS. NCDs, non-communicable diseases.

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Th-cell function, determines susceptibility to allergic disease. This results in altered gene expression, differential capacity to generate Th-cell subtypes, and altered immune responses following cell activation.

BIS Microbiome

The objective of BIS Microbiome is to conduct a longitudinal investigation of the relationship between the composition and metabolic products of the maternal and infant gut microbiome, immune development (including epigenetic profiles) and allergic disease, asthma and atherosclerosis. Recent evidence in animal models has shown that dietary factors may modify the risk of allergic disease and asthma via changes in the large bowel microbiome and the production of short chain fatty acids (SCFAs), which in turn have clearly delineated effects on the local and systemic immune system.[8,9] It has also been shown that *Clostridia* regulate innate lymphoid cell function and intestinal permeability to protect against allergen sensitization.[10] These findings may be of substantial public health significance, but human studies are now required. Our initial aims are to test the hypotheses that a faecal profile at 1 month of postnatal age characterized by (i) low diversity, (ii) low *Clostridia* and (iii) low SCFAs is associated with an increased risk of IgE-mediated food allergy at age 1 year.

BIS Immune

The objective of BIS Immune is to evaluate the relationship between pre- and postnatal microbial exposure, the large bowel microbiome and its metabolites, vitamin D status (VDS), early-life immune development and risk of allergic disease, asthma and other NCDs. Recent studies have suggested that increased regulatory T (Treg) cells may contribute to reduced risk of allergic disease and asthma among children from farming environments.[11] There is also emerging evidence that low VDS may be associated with an increased risk of allergic outcomes and that this relationship may be modified by genetic and or environmental factors.[12] Our initial aims are to test the hypotheses: that a reduced proportion of Treg cells in peripheral blood during early infancy is associated with an increased risk of food allergy; that the deficit in Treg relates to the composition and metabolic products of the large bowel microbiome; and that the relationship between VDS and food allergy is modified by the composition of the large bowel microbiome.

BIS Respiratory

The objective of BIS Respiratory is to investigate the relationship between postnatal immune development, lower respiratory tract illness, aeroallergen sensitization and deterioration in lung function during the first 3 years of life.[13–15] Early-life respiratory development can have a substantial impact on long-term respiratory health.[16] Early-life immune dysregulation is associated with an increased risk of both aeroallergen sensitization and febrile lower respiratory tract infections during the first years of life.[13] In turn, aeroallergen sensitization and febrile lower respiratory tract infections during early life are associated with an increased risk of asthma in later childhood.[14] Both processes result in respiratory inflammation during a critical period of lung development.[15]

BIS Cardiovascular

The objective of BIS Cardiovascular is to conduct a comprehensive longitudinal investigation of factors contributing to the development of the inflammatory process of atherosclerosis and of other cardiovascular disease (CVD) risk factors, from early life onwards. Current preventive strategies for CVD target adults, but the underlying pathology of CVD—atherosclerosis—often begins *in utero*[17] and progresses for decades before becoming clinically apparent.[18] Early life is therefore a critical period for the initiation and progression of atherosclerosis but is a largely overlooked window of opportunity for prevention. This is the first study to investigate aortic intima-media thickness (IMT) longitudinally from birth into early childhood and its relationship with other cardiovascular phenotypes. Our initial aim is test the hypotheses that perinatal inflammation is associated with increased aortic intima-media thickness at 1 month of age, and that early-life infection burden is associated with an adverse cardiovascular risk in pre-school children.

BIS Neurodevelopment

The objective of BIS Neurodevelopment is to investigate whether higher levels of exposure to specific modern chemicals are associated with deficits in validated measures of executive function, memory, behaviour, language and general cognitive development during the first years of life. There has been a well-documented increase in the burden of disorders of development and behaviour within paediatrics.[19] The environmental factors contributing to this increase are unknown, but there is considerable concern regarding the role of modern environmental chemical exposures.[20] The array of biospecimens collected in BIS provides a unique opportunity to assess the association between exposure to specific modern chemical exposures in early life and adverse neurodevelopment. The potential neurotoxicants currently under investigation include bisphenol A (BPA) and phthalates. The relationship between these exposures and immune development will also be explored.

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Table 1. Eligibility criteria

Inclusion criteria	Exclusion criteria
Eligible women were: 1. Residents of the Barwon Statistical Division (geographically defined region) 2. Pregnant at no more than 28 weeks of gestation at the time of enrolment 3. Planning to give birth at either Geelong Hospital (public) or St John of God Hospital (private) 4. Intending to be available for the duration of the study	Women were excluded if they: 1. Were not an Australian permanent resident 2. Were unable to complete questionnaires without the assistance of an interpreter 3. Were unable to give informed consent for various reasons and no third party could be identified or, informed consent was not given 4. Were under the age of 18 years at the time of maternal blood sample collected at 28 weeks of pregnancy 5. Were previous participants of the Barwon Infant Study with at least one live-born child already included in the cohort 6. Were planning to pay to have their infant's cord blood stored privately for future use[a] 7. Had moved out of the Barwon Statistical Division by the time the baby was born Infants were excluded if they: 1. Were born before 32 completed weeks of gestation 2. Had a serious illness, identified during the first few days of life 3. Had a known major congenital malformation or genetically determined disease

[a]Private cord blood banking is currently uncommon in Australia.

Who is in the sample?

The Barwon region, which includes and surrounds the city of Geelong, has a range of primary industries and incorporates metropolitan, rural and coastal areas. The population characteristics are similar to those of the Australian population overall, with the exception that there is a smaller proportion of families from non-English-speaking backgrounds. Approximately 260 000 people live in the Barwon region and there are about 3000 live births per year. More than 90% of these occur at either Geelong Hospital (a government-funded healthcare facility) or St John of God Hospital (a private facility). At both hospitals pregnant women have an antenatal book-in appointment at approximately 15 weeks of pregnancy, and women attending this antenatal clinic were invited to participate in BIS. The eligibility criteria are shown in Table 1. The 3-year recruitment phase was completed in June 2013 ($n = 1158$ women). The baseline characteristics of the participating infants ($n = 1074$) are shown in Table 2. From monitoring the attendance of over 1000 women at the 15-week hospital book-in appointments, more than 90% of attendees reported meeting the self-assessed eligibility criteria, and among those invited to participate, our recruitment rate was approximately 33%. Focus group work indicates that this relates to the high participant burden and the bio-intensive nature of the protocol. A comparison of participating mothers and non-responders is shown in Table 3. For certain analyses we will examine potential sensitivity of results to participation bias by weighting observed data by the inverse of the estimated probability of participation,[21] with these estimates obtained as predicted values from a logistic regression of participation on sex, maternal age, household size and socioeconomic status by postcode.

How often are cohort members being followed-up?

There have been frequent, and ongoing, participant contacts during pregnancy and the first postnatal years (Table 4). The 4-year reviews commenced in late 2014 and the follow-up schedule beyond 4 years are yet to be finalized. The retention rate for the 1-year reviewwas 894 of 1074 eligible infants (83.2%) (Figure 2). Participants who had not completed the 1-year review were more likely to have been born in the government-funded hospital and to have a lower birthweight; their parents were more likely to be younger and to have a lower income; and their mothers were less likely to have attended tertiary education and were more likely to have smoked cigarettes or been exposed to passive smoke during pregnancy (Table 5).

What has been measured?

Wherever possible we have used questionnaire items for which validity has been examined and established. Questionnaires have been developed to facilitate pooling of data with other birth cohort studies being conducted in Australia (such as the HealthNuts study in Melbourne[22]) and internationally. In particular, BIS is a member the International Childhood Cancer Cohort Consortium (I4C)[23] and the International Inflammation Network (in-FLAME). The questionnaire domains and timing are shown in Table 4. The schedule of biosample collection and funded assays during pregnancy and the first 3 years of life is shown in Table 6. The schedule of physical, physiological and clinical measurements is shown in Table 7.

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Table 2. Baseline characteristics of the 1074 eligible infants

Characteristics	Inception birth cohort ($n = 1074$)
Twins	20 (10 pairs) (1.9%)
Sex of child: male	556 (51.8%)
Maternal age, years (mean and standard deviation)	32.1 (4.8)
Paternal age, years (mean and standard deviation) $n = 1013$	34.2 (5.8)
Maternal level of education:	
Less than year 10 of high school	12 (1.1%)
Year 10 of high school equivalent	77 (7.3%)
Year 12 of high school equivalent	161 (15.2%)
Trade, certificate or diploma	253 (23.9%)
Bachelor degree	349 (33.0%)
Postgraduate degree	190 (18.0%)
Other	16 (1.5%)
Delivered in a publicly owned (government) hospital	775 (72.8%)
Household income (gross, Australian dollars per annum):	
Less than $25,000	26 (2.5%)
$25,000 to $49,999	99 (9.3%)
$50,000 to $74,999	184 (17.3%)
$75,000 to $99,999	263 (24.8%)
$100,000 to $149,999	340 (32.0%)
More than $150,000	119 (11.2)
Unsure or declined to answer	30 (2.9%)
Number of siblings:	
0	449 (42.2%)
1	378 (35.5%)
2	182 (17.1%)
3 or more	55 (5.2%)
Maternal cigarette smoking:	
3 months prior to conception:	
None	885 (84.5%)
1–10 per day	103 (9.8%)
11–20 per day	43 (4.1%)
>20 per day	16 (1.5%)
During first trimester:	
None	949 (90.4%)
1–10 per day	80 (7.6%)
11–20 per day	17 (1.6%)
>20 per day	4 (0.4%)
During second trimester:	
None	984 (93.7%)
1–10 per day	54 (5.1%)
11–20 per day	11 (1.0%)
>20 per day	1 (0.1%)
Passive smoking (during preconception or pregnancy)	182 (17.1%)
Pet ownership	781 (73.8%)
Livestock ownership	73 (7.0%)
Family history in a first-degree relative of:	
Asthma	539 (51.6%)
Hay fever	668 (64.4%)
Eczema	475 (45.8%)
Delivery via caesarean section	324 (30.5%)
Gestational age at birth:	
32 to 36 completed weeks	43 (4.0%)
37 to 42 completed weeks	1021 (96.0%)
> 42 completed weeks	0 (0.0%)
Birthweight in grams (mean and standard deviation)	3530 (525)

Assessment of eczema status

The presence of eczema is defined using the Williams UK diagnostic criteria.[24] The severity of eczema is assessed using the SCORAD.[25] Atopic eczema is defined as the presence of eczema plus allergic sensitization (as demonstrated by a positive skin-prick test to one or more allergens).

Assessment of allergic sensitisation

Skin-prick allergy testing (SPT) is performed according to standard guidelines.[26] A positive skin-prick test is defined as a wheal diameter at least 2 mm greater than that produced by a negative control solution, measured at 15 min. We perform SPT using Quintips®to the following allergens: cow's milk, egg, peanut, sesame, cashew, dust mite (*Dermatophagoides pteronyssinus* 1), cat, dog, rye grass and *Alternaria tenius* (Hollister Stier, Aloystal, ALK®).

Assessment of IgE-mediated food allergy

Infants who exhibit a positive SPT to any of the five foods tested are invited to attend a BIS food allergy clinic for clinical assessment and/or oral food challenge. Infants with a clear history of an immediate-type reaction following exposure to a specific food to which they are skin-prick positive are classified as allergic to that food. In the absence of such history, oral food challenges for egg, peanut, sesame and cashew are undertaken regardless of the SPT wheal size,[22] using standardized food challenge protocols.[27] A positive food challenge is defined according to the protocol established by the HealthNuts study.[28]

Assessment of lung function

Multiple Breath Washout (MBW) is a recently validated technique for measuring lung function, which is available for use in infants during natural (unsedated) sleep.[29] The technique measures the degree of ventilation inhomogeneity (inefficient breathing), which has emerged as an important feature of early respiratory disease processes.[30] In individuals with asthma, ventilation inhomogeneity reflects the degree of peripheral airway obstruction and is a major determinant of airway responsiveness.[32]

Assessment of cardiovascular risk in early life

The distal aorta is the optimal site to assess early markers of atherosclerosis in infancy.[33] Aortic IMT is a validated measure of preclinical atherosclerosis in childhood, but one that has only recently been applied to newborns.[34] The extent of atherosclerosis in the aorta correlates with the extent of atherosclerosis in the coronary arteries in later life ($r = 0.37$, $p = 0.001$).[35] In both unselected children and in those at increased risk of NCDs (e.g. diabetes or hypercholesterolaemia), aortic IMT is actually a more sensitive measure of atherosclerosis than carotid IMT.[36–38] We will

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Table 3. Comparison of participating mothers and non-responders on baseline characteristics

Characteristics	Participants ($n = 1064$)	Non-responders ($n = 2869$)
First-degree relative with asthma	539/1059 (50.9%)	506/1600 (24.1%)
First-degree relative with eczema	475/1057 (44.9%)	362/2094 (17.3%)
Socioeconomic index tertiles for area code		
Low SEIFA	259 (25.6%)	772 (32.3%)
Medium SEIFA	194 (19.2%)	466 (19.5%)
High SEIFA	557 (55.1%)	1154 (48.2%)
Remoteness classification for area code		
Urban	276 (27.1%)	604 (25.1%)
Suburban	742 (72.8%)	1798 (74.7%)
Rural	1 (0.1%)	4 (0.2%)
Number of people living in the family home		
1 person	12 (1.1%)	17 (0.8%)
2 people	410 (38.9%)	800 (38.5%)
3 people	363 (34.5%)	698 (33.6%)
4 people	199 (18.9%)	357 (17.2%)
5 or more people	69 (6.6%)	207 (9.9%)

SEIFA, socioeconomic index tertile for area.

Table 4. Schedule of participant reviews and questionnaire domains

Domains	28-week antenatal	Birth	1 month	3 months	6 months	9 months	1 year	18 months	2 years	4 years
Nature of contact:										
Physical review	+	+	+		+	+	+		+	+
Questionnaires	+	+	+	+	+	+	+	+	+	+
Mother's health	+		+							
Mother's mental health	+		+		+		+		+	+
Mother's medication use	+		+		+		+		+	
Parental characteristics	+									
Family medical history	+									
Demographic & SES measures	+		+				+		+	
Pets/livestock	+		+				+		+	+
Parental lifestyle	+		+		+		+		+	+
Breastfeeding	+	+	+	+	+	+	+			
Child's diet			+	+	+	+	+	+		+
Food reactions			+	+	+	+	+	+	+	+
Child's eczema symptoms			+	+	+	+	+	+	+	+
Child's respiratory health			+	+	+	+	+	+	+	+
Child's other illnesses			+	+	+	+	+	+	+	+
Child's health resource utilization			+	+	+	+	+	+	+	+
Child medications			+	+	+	+	+	+	+	+
Sibling health			+		+		+		+	+
Pesticide exposure	+		+		+	+	+	+	+	+
Household chemical exposure	+		+		+	+	+		+	+
Parental sun exposure	+									
Child sun exposure			+		+		+		+	+
Child care			+		+		+	+	+	+
Child's sleeping			+		+		+	+	+	+
Household heating/cooling			+			+	+		+	+
Child's hygiene			+		+		+		+	+
Child's behaviour and neurodevelopment						+		+	+	+
Parenting practices						+			+	
TV/screen time							+		+	+

SES, socioeconomic status.

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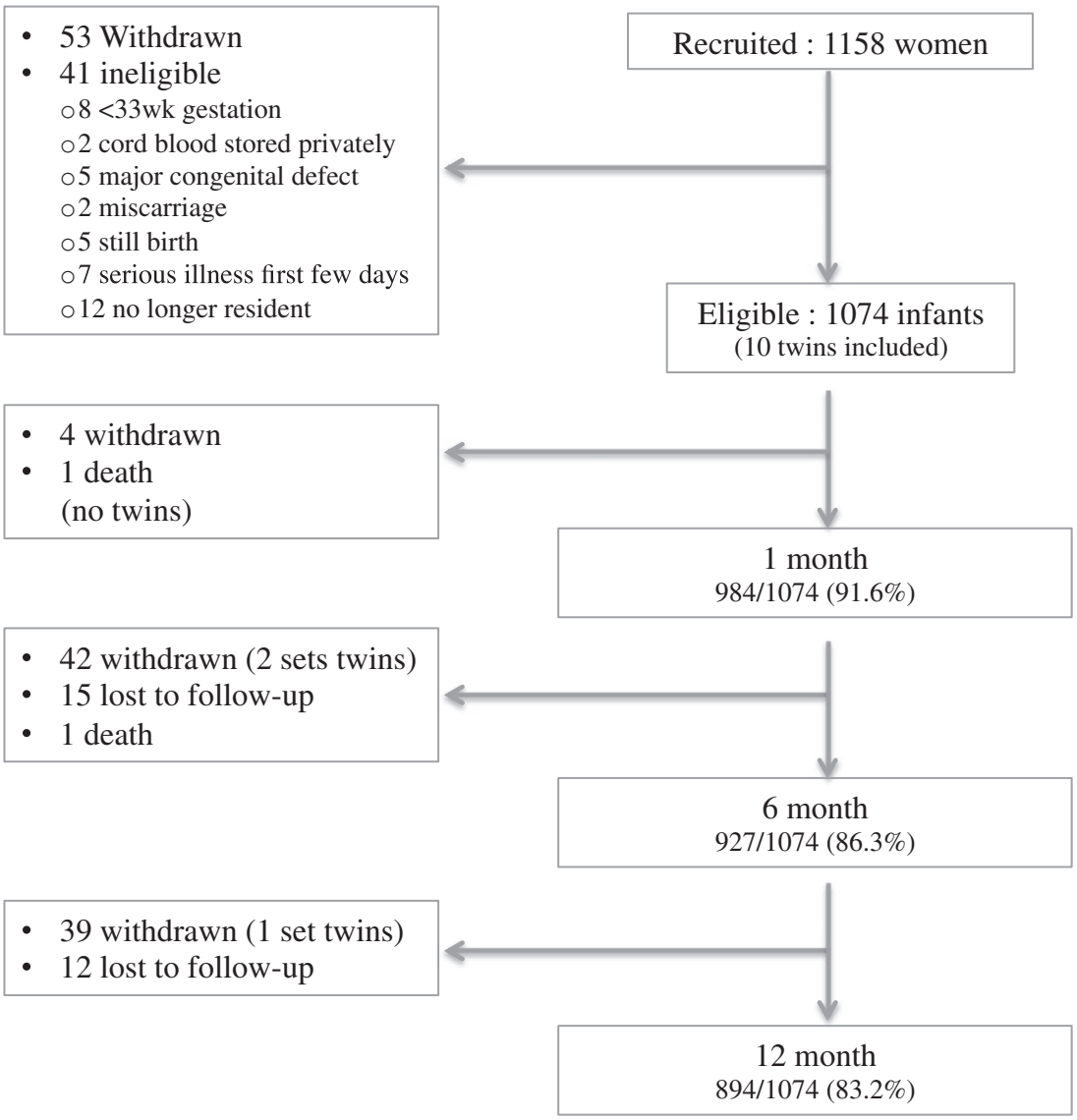

Figure 2. Retention data among the inception birth cohort ($n = 1074$).

also measure blood pressure at all ages and, additionally, carotid IMT and pulse wave velocity (a validated measure of arterial elasticity) at 3 years of age.

Assessment of neurodevelopment

At 9 months we conduct direct measurements of executive function and processing speed,[39,40] in combination with parent-reported measures of global development[41] and temperament.[42] At 18 months early language development is assessed by questionnaire.[43] At 2 years we conduct a direct measurement of cognitive, language and motor development using the Bayley Scales of Infant and Toddler Development (third edition),[44] in combination with the Child Behaviour Checklist.[45] Executive function is again assessed at 4 years.

What has it found? Key findings and publications

We have published review and or hypothesis papers regarding a number of the investigations in BIS,[46–48] described the potential role of cord blood flow cytometry as a population screening strategy[49] and reported the performance of baseline measurement techniques.[50] Recruitment and definition of the BIS inception cohort was completed in December 2013 and numerous manuscripts are in preparation.

Maternal folate levels during pregnancy

Sufficient folate status prior to conception and through the first trimester of pregnancy is recommended to reduce

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Table 5. Comparison of baseline characteristics on retention to 1 year

Characteristics	Completed the 12 month review	
	Yes ($n = 894$)	No ($n = 180$)
Twins	14 (7 pairs) (1.6%)	6 (3 pairs) (3.3%)
Sex of child: male	463 (51.8%)	93 (51.7 %)
Maternal age, years (mean and standard deviation)	32.6 (4.5)	29.5 (5.2)
Paternal age, years (mean and standard deviation) $n = 1013$	34.6 (5.6)	32.5 (6.8)
Maternal level of education:		
Less than year 10 of high school	6 (0.7%)	6 (3.4 %)
Year 10 of high school equivalent	40 (4.5%)	37 (21.3%)
Year 12 of high school equivalent	123 (13.9%)	38 (21.8%)
Trade, certificate or diploma	219 (24.8%)	34 (19.5%)
Bachelor degree	315 (35.6%)	34 (19.5%)
Postgraduate degree	168 (19.0%)	22 (12.6%)
Other	13 (1.5%)	3 (1.7%)
Delivered in a publicly owned (government) hospital	618 (69.7%)	157 (88.7%)
Household income (gross, Australian dollars per annum):		
Less than $25,000	13 (1.5%)	13 (7.5%)
$25,000 to $49,999	66 (7.4%)	33 (19.0%)
$50,000 to $74,999	154 (17.4%)	30 (17.2%)
$75,000 to $99,999	228 (25.7%)	35 (20.1%)
$100,000 to $149,999	303 (34.2%)	37 (21.3%)
More than $150,000	102 (11.5%)	17 (9.8%)
Unsure or declined to answer	21 (2.4%)	9 (5.2%)
Number of siblings:		
0	372 (41.9%)	77 (43.5%)
1	315 (35.5%)	63 (35.6%)
2	158 (17.8%)	24 (13.6%)
3 or more	42 (4.7%)	13 (7.3%)
Maternal cigarette smoking:		
3 months prior to conception:		
None	769 (87.9%)	116 (67.4%)
1–10 per day	74 (8.5%)	29 (16.9%)
11–20 per day	23 (2.6%)	20 (11.6%)
>20 per day	9 (1.0%)	7 (4.1%)
During first trimester:		
None	820 (93.3%)	129 (75.4%)
1–10 per day	46 (5.2%)	34 (19.9%)
11–20 per day	11 (1.3%)	6 (3.5%)
>20 per day	2 (0.2%)	2 (1.2%)
During second trimester:		
None	846 (96.2%)	138 (80.7%)
1–10 per day	29 (3.3%)	25 (14.6%)
11–20 per day	3 (0.3%)	8 (4.7%)
>20 per day	1 (0.1%)	0 (0.0%)
Passive smoking (during preconception or pregnancy)	136 (15.3%)	46 (26.0%)
Pet ownership	651 (73.6%)	130 (74.7%)
Livestock ownership	62 (7.1%)	11 (6.4%)
Family history in a first-degree relative of:		
Asthma	442 (50.6%)	97 (57.1%)
Hay fever	573 (65.9%)	95 (56.9%)
Eczema	405 (46.8%)	70 (40.5%)
Delivery via caesarean section	278 (31.3%)	46 (26.3%)
Gestational age at birth:		
32 to 36 completed weeks	36 (4.5%)	7 (4.1%)
37 to 42 completed weeks	851 (95.9%)	170 (96.0%)
> 42 completed weeks	0 (0.0%)	0 (0.0%)
Birthweight in grams (mean and standard deviation)	3547.6 (524.0)	3442.4 (527.2)

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Table 6. Schedule of biospecimen collections and currently funded assays

Specimens	28-week antenatal	Birth	1 month	6 months	9 months	1 year	2 years	4 years
Blood	Maternal	Cord blood		Child		Child		Child
Funded assays/ measures	Vitamin D, lipid profile, CRP, endotoxin, red cell folate	Vitamin D, flow cytometry, IFNy mRNA response capacity and gene methylation		Vitamin D, flow cytometry, IFNy response capacity and gene methylation		Vitamin D, flow cytometry, IFNy response capacity and gene methylation		Lead
Faeces	Maternal	Meconium	Child	Child		Child	Child	Child
Funded assays/ measures			Short chain fatty acids					
Saliva				Maternal & child		Child		
Anterior nasal swab		Child	Child	Child		Child		Child
Urine	Maternal		Child		Child	Child		
Funded assays/ measures	Cotinine, Pthalates, Bisphenol A							
Hair			Maternal & child					
Breast milk			Child					

CRP, C-reactive protein; IFNy, interferon gamma; mRNA, messenger ribonucleic acid.

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Table 7. Schedule of physical, physiological and clinical measurements

Measurements	28-week antenatal	Birth	1 month	6 months	9 months	1 year	2 years	4 years
Lung function			Child					Child
Transabdominal ultrasound (aortic intima-media thickness)			Child					Child
Blood pressure			Child					Child
Carotid intima-media thickness								Child
Pulse-wave velocity								Child
Ultrasound measurement of fetal growth	Maternal & child							
Eczema status			Child	Child		Child		Child
Skin-prick allergy testing						Child		Child
Formal food challenge among sensitized infants						Child		Child
Height, weight & head circumference	Maternal & paternal	Child	Maternal	Child		Child	Child	Child
Skinfold thickness		Child	Child	Child		Child	Child	Child
Fagan test of Infant Intelligence					Child			
A-not-B task					Child			
BAYLEY-III developmental assessment							Child	
Child Behaviour Checklist for Ages $1^1/_2$–5							Child	
Executive function tasks								Child

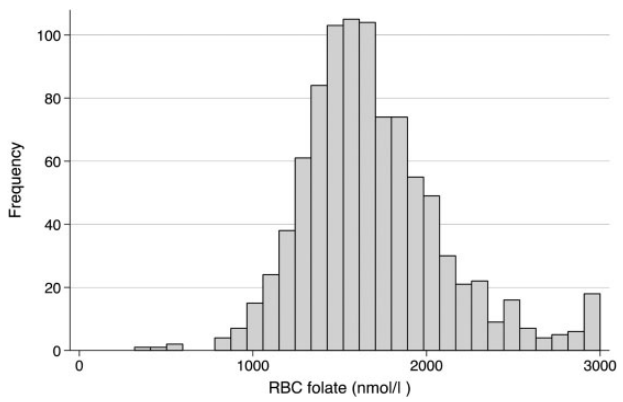

Figure 3. The distribution of maternal red blood cell (RBC) folate among BIS mothers ($n = 939$) at 28 to 32 weeks of pregnancy.

the risk of neural tube defects (NTDs) and other major congenital malformations.[51] In this context, folic acid food fortification programmes have been introduced in many parts of the world—including Australia in 2009. Women are also advised to increase folate intake during pregnancy.[52] Of potential concern, folic acid in pregnancy has been linked to epigenetic changes associated with an increased risk of allergic disease and asthma,[53] although the evidence from human cohort studies is conflicting.[54] The implementation of mandatory fortification of flour with folic acid has been associated with a reduced prevalence of folate deficiency among Australian women of childbearing age,[55] but there are no recent data regarding maternal folate levels during pregnancy. A red blood cell (RBC) folate level of greater than approximately 900 nmol/l is considered sufficient to reduce the risk of NTDs.[51] Although there is little evidence to guide the upper limit of the desired range, previous studies have used a threshold of 2000 nmol/l;[55] 998 of 1064 (93.8%) women reported taking supplements or multivitamins containing folic acid during the first and second trimesters of pregnancy. RBC folate was measured at 28 to 32 weeks of gestation in 939/1064 (88%) mothers. The mean (standard deviation) RBC folate was 1693 (95% confidence interval 1667 to 1720) nmol/l; only 10/939 (1.1%) of women had a level <900 nmol/l whereas173/939 (18.4%) had a level >2000 nmol/l (Figure 3). These levels are higher than have been reported in previous population-derived cohorts of pregnant women.[56]

What are the main strengths and weaknesses?

The major strengths of BIS are the population-derived antenatal sampling frame, in combination with a highly detailed array of longitudinally assembled biospecimens and physiological/clinical measures. The majority of previous and contemporary birth cohort studies are: either (i) substantially larger than BIS but have a much less extensive schedule of biospecimen collection and physiological measures (depth of phenotyping); or (ii) involve a similar level of participant burden to BIS, but are undertaken among smaller cohorts focused on particular phenotypic outcomes. The population-derived design of BIS will enable us to address a range of outcomes, and facilitates a nested case-cohort approach to research questions where the biospecimen processing and analysis are resource-intensive;

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for example, studies involving isolation and detailed characterization of specific cryopreserved mononuclear cell populations. A nested case-cohort approach can provide a similar level of rigour and statistical power to using the entire cohort for addressing resource-intensive research questions, but is far more efficient in terms of both cost and biosamples.

The detailed protocol poses high participant burden, so optimizing participation and attrition rates among the unselected cohort is an important challenge. The Barwon setting is well suited to this approach, as it is serviced by a single obstetric and paediatric network and enjoys a strong sense of community, while also encompassing a substantial population.

The research agenda has evolved to incorporate a number of different health outcomes that have origins in early life and which are believed/hypothesized to share many environmental and immunological determinants. It has been designed to foster interdisciplinary synergies and cross-pollination between different lines of investigation. For example, the detailed description of gut microbiota and immune development in the BIS Immune component are likely to be relevant to the initiation and potentiation of the inflammatory process under investigation in BIS Respiratory and BIS Cardiovascular, respectively.

There are limitations to the study. By international standards, a sample of 1074 infants is a relatively small cohort, and this will limit our capacity to investigate uncommon outcomes and to perform exploratory genomic association studies, especially for dichotomous phenotypes. We have judged that, in view of the high participant burden, it would be difficult to maintain an adequate retention rate among a larger, multi-centre cohort. Our participation fraction was approximately 33%, which may detract from the population representativeness of the cohort but is unlikely to introduce substantial bias in estimates of exposure-disease associations.[57]

These limitations are, however, offset by the detailed longitudinal data and biological specimens, as well as physiological and clinical measurements. The capacity of epidemiological studies, including cohorts, to incorporate molecular biology and 'omics' has recently been discussed in depth.[1] With this in mind, BIS has been designed to achieve a balance between statistical power, deep phenotyping and a manageable level of participant burden.

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

Further information about BIS can be obtained via the BIS website: [www.barwoninfantstudy.org.au] or by e-mailing [peter.vuillermin@deakin.edu.au]. Requests for access to the data or biosamples and establishment of collaborative projects are considered by the BIS Steering Committee.

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Conflict of interest: Mimi Tang is on the medical advisory board (Oceania) Nestle Nutrition Institute; the medical advisory board (Australia New Zealand) Nutricia; Global scientific advisory board immunology allergy Danone Nutricia; and has received speaker fees from Danone Nutricia.

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